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SCIENCE & TECHNOLOGY POLICY

New Steps in S&T Management Reform Urged

[Article by Song Jian [1345 0256]: "Make New Strides in Reforming the Science and Technology System"]

[Text] I. The Process of Reforming the Science and Technology System

Improving or creating labor production capabilities and raising national per-capita output constitute China's general strategy for the new historical period. The 13th Central Committee has specified that the central task of the science and technology sphere is to orient itself to the economy and to help realize the overall strategic objectives.

China's scientific and technical capabilities are not inferior to those of many mid-level developed countries. In the 1950's and 1960's, the appearance of the "two bombs and one satellite," the successful synthesis of insulin and the like were truly representative contributions by China's scientists that revealed a superior scientific and technological level. In addition, the great achievements on all fronts in China's economic construction were an embodiment of the painstaking effort and exertion of China's scientists and its multitude of scientific and technical personnel. But it must be admitted that China's scientific and technical activities have long been out of close touch with economic development and they have not been able to make a vigorous effort to increase China's per-capita labor productivity. China's 1987 per-capita national output was not quite 1,000 yuan and was equivalent to only slightly more than $300 in international market exchange value; this figure is only a tenth as great as those for certain Eastern European countries and is only a few percent of the figures in the developed countries. This is a concentrated expression of China's inadequate economic development. It was under these circumstances that the party Central Committee enunciated the general guidelines for scientific and technical reform, specifying that all possible methods should be used to bring the scientific and technical sphere into the economic domain and make it serve economic construction and strive to increase the labor productivity of the entire people. In the early 1980's, under the direct guidance of the party Central Committee and the State Council, channels linking the scientific and technical departments to the economy were gradually opened in order to bring science and technology into the economy. Some results have already been realized in the ensuing years.

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--Opening Up of Technology Markets. The technology market has made science and technology begin to take root in the economy, has organically linked supply and demand mechanisms, and has accelerated the utilization of scientific and technical results and their conversion to commodity production. Transactions on technology markets last year totaled 3.35 billion yuan, and the average annual increase during the past 3 years has been 46 percent. In the future, the technology market will become increasingly regularized, and the holding of regular trade fairs in certain major cities will become established, constituting a trend in science and technology markets.

--Implementation of the Spark Plan. The Spark Plan, intended to develop a commodity economy with science and technology as its mainstay, has already begun to set the tone for vitalization of the rural economy. In the past, some research institutes established on the three fronts were unaware of everyday life and unconcerned with what went on outside their walls. But all around us live our dear peasantry, thirsting for the blessings of science and technology. The Spark Plan changed the situation, and now nearly 400,000 scientific and technical personnel have gone to the countryside to bring science and technology to the peasants. Every year the Spark Plan helps the peasants to establish more than a thousand demonstration projects; the annual investment is 2.5 billion yuan; and every year it gives practical technical training to a million persons. Young peasants armed with science and technology are boldly challenging traditional conceptions, and are scientifically establishing new forms of labor and production, which have greatly increased labor productivity. A large number of new types of able producers, and types of wealth creation have already emerged among the peasantry, and many of the most attractive among them have been chosen as National People's Congress delegates.

--Reform of the Funds Allocation System. Reform of the science and technology funds allocation system opened up multilevel, multichannel sources of funding for scientific research. In 1987, the total lateral earnings of independent scientific and technological bodies and advanced academies and schools exceeded annual allocations for scientific research by the state finance organs. Last year's lateral earnings of development bodies under 57 ministries and commissions were 159 percent as great as scientific research allocations by the financial organs.

--Promoting a Union of Science and Technology With the Economy and Establishing Various Types of Science and Technology Enterprises. The various types of integrated bodies linking science and technology with the economy that have emerged from practice already number more than 10,000. Last year more than 100 national and province-level independent scientific research organizations joined enterprises or enterprise groups. Some large academies, institutes and advanced schools headed economic entities or took the lead in establishing pathbreaking enterprise collectives engaged in scientific research, or set up foreign-oriented enterprises or joint ventures; and some developed into technological development centers for specific industries or localities, oriented toward medium and small-size enterprises or small-town enterprises. The number of science and technology enterprises implementing various ownership systems has already surpassed 10,000. They used flexible

operating and management systems, engage in their own fund-raising, carry on independent operations, take responsibility for their own profits and losses, and integrate technology, industry and trade, and they have proved to be quite vigorous.

--The masses have already become aware of the effects and influences of science and technology, which in particular have won the support of the 800 million peasants. A large contingent of scientific and technological cadres have already been sent to the countryside, not for "reeducation," but to contract for agricultural production and to head small-town enterprises; some have taken the posts of county heads [of science and technology committees], deputy county heads, district directors or assistant directors. They are not included in the regular complement of cadres in these locations, but they help the local cadres, using science and technology to guide the emergence and development of technology at the rural level and to help vitalize the rural economy. Their activities have been warmly welcomed by the peasants, who have stated, "Sending science and technology cadres is better than sending money or materials." In the next 1 or 2 years, most counties nationwide will select deputy county heads for their science and technology committees.

II. Problems Arising in the Course of Reform

In the last 3 years, under the impetus of the reform of the science and technology system, the operations system and structure of science and technology activities have undergone great changes. But overall, the effect of scientific and technological activities in promoting the development of the socialist commodity economy has been far from sufficient. A primary indication of this fact is that more than 100 large academies and advanced schools under the various departments of the State Council are still experiencing considerable difficulty in promoting economic construction, the establishment of high-technology industries has not yet achieved breakthrough status or begun to set a tone, and has not yet become a support of the national economy. Only one-third of these organizations have full employment, while the other two-thirds of their personnel have no work or are not being fully utilized. As Comrade Zhao Ziyang pointed out, we have started out by waging a 3-year guerrilla war, but the main forces have not yet been committed, and the results of reform in these localities have been very small. Many factors have contributed to this situation; some of them are problems of awareness, while others involve the economy and the science and technology system.

A. Our major academies, institutes and advanced schools are actually tackling rather numerous and advanced new technologies and high technologies in a wide range of technological fields. But owing to the smallness of the domestic new-technology and high-technology market, the inability of most enterprises to assimilate technologies, and the lack of a capacity to develop into national markets, new technology and high technology have had difficulty in producing an effect in the economy. The problem constantly raised by scientific and technical personnel is this: What can we do when we are ready to sell and there is nobody to buy, when we are ready to orient ourselves, but nobody is ready to rely on us?

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B. Strictly speaking, China's commodity economy is in its infancy, and a modern technology market has not yet taken shape; in particular, the high-technology market is greatly dependent on the opening up of international markets. China's commodity economy is not yet sufficiently developed, and the idea of using science and technology to develop the economy and the mechanism for doing so are far from being fully established. Owing to an inadequate development of the commodity economy, and in particular the new technology and high technology areas, as well as to the limited number of domestic markets, an inadequate ability to develop international markets, high technology is not profitable and it is impossible to obtain compensation exclusively through technology transfer. In the advanced countries, as soon as word of a new technological development comes out, many enterprises immediately beating a path to the developer's door in the hope of buying the technology or process. In China, the inventors of a new product or technology have no recourse but to ask the government for money; this example is a clear indication that China's commodity economy is still inadequately developed.

C. Some comrades still have a lingering fondness for the previous system and hope to return to the old path. They hope to restore the methods of the 1950's, when research projects were all handed down from the leadership organs and when they were always hoping that some day a glorious major task would fall on their shoulders, so that they would be able to perform research and make a contribution with the support of state allocations. These comrades lack the conviction and spirit to involve themselves in the struggle on the front line of the market economy. Of course, in the future some research tasks certainly will be handed down by the state, but overall, as the commodity economy develops, such chances will become fewer and fewer.

D. Specification of the direction and ultimate model for the development of development organizations requires a period of exploration and finding out. So long as people are not entirely clear about the long-term objectives of the reform, this will hinder the reform of research institutes. For a certain period, some research institutes will not be greatly enthusiastic about the commodity economy and will lack the farsightedness to engage in large-scale industries and large-scale development, but will generally get by on short-range projects or will even be satisfied so long as their earnings are sufficient to provide bonuses. Although they will have access to high technology, they will be able to find only low-grade projects. For example, in electronics technology, many large, high-level research institutes would be capable of developing any imaginable type of high-level electronics product, but high-technology products are not marketable and no one is working on products that would be marketable. Not only are such highly marketable products as color televisions and VCR's in short supply domestically, but some European countries are buying all that are available. Several dozen percent of the parts and components of our color televisions are still imported, but very few of the research institutes are making an effort on this problem. Thus our top-flight personnel and excellent technology are being tied up.

III. New Ideas on Reform

Based on changes in the current world economic situation, the party Central Committee has decided to implement the policy of economic development of the coastal regions. In order to implement the Central Committee's strategic plan, practically all of China's 18,000 km of coastline will have to be opened up, and nearly 200 million people will be directly involved in establishing and developing export-oriented labor-intensive technology-intensive industries. This provides a great opportunity for our new-technology and high-technology development capabilities to enter the economy. We must seize the opportunity, make prompt decisions, and guide China's new technology and high technology toward international markets. We must make an organized effort to engage the effective strength of the large academies and institutes, advanced schools and large and medium-sized enterprises on the front line of the coastal economy and have them make a contribution to the development of an externally oriented economy there. As a result, this year the party Central Committee has instituted a new policy and a new plan for the reform of the science and technology system.

A. While implementing the coastal economic development strategy, we must go all out to establish and develop new-technology and high-technology industries oriented to international markets. In developing high technology, we must start by developing export-oriented high-technology industries. If these industries are not developed, we will not be able to reap large economic benefits. New-technology and high-technology industries necessarily can rely only on international markets and develop products that are salable on such markets. No country's high-technology industry can maintain itself exclusively by the domestic market; if it does not orient itself to international markets and win a share of them, it will lose its viability.

B. Encourage large academies and institutes, advanced schools, and large and medium-sized enterprises to run companies, enterprises, institute branches and showrooms in the coastal area, or join up with medium and small-size enterprises and small-town enterprises in order to set in motion the development of an externally oriented economy there. When attempting to develop in the coastal region, they must focus on utilizing their special strengths. According to the economic principle of relative superiority, every country necessarily has relatively superior products that no other country has, that it makes better and more cheaply and can import in large amounts. The decisive factor is the search for the optimum combination of resources, manpower, skills and funds. Making thorough use of points of superiority is the only way to augment one's development potential in this kind of activity and establish oneself on the international market.

C. Developmental research organizations must lead the new wave in economic activity. They must make full use of scientific and technical superiority, and strive to develop enterprise groups pioneering in modern technology. If China's more than 100 large academies and institutes could produce 500 enterprise groups to pioneer in science and technology and to get them into the international market, they would be able to make a splendid contribution to China's economic development. A group of development-oriented research

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organizations that had the prerequisites could also develop into international companies. The result would be not only that science and technology would be oriented toward the economy and would grow into the economy, but in addition that they would be able to accumulate funds on international markets, pave the way for even greater development of science and technology in China, and greatly improve their own earnings. To summarize, organizing, developing toward enterprise groups pioneering in science and technology, and proceeding toward international markets constitute an approach and a solution for the growth of development organizations.

D. To develop externally oriented industries, we must train a large contingent of entrepreneurs of a new type who understand science and technology as well as operations and who are a match for foreigners on international markets. Scientific and technical entrepreneurs are the organizers and creators of the newest productive forces, and the optimum combination of science and technology, funds, personnel and markets will be implemented by them. Unless a large contingent of scientific and technical entrepreneurs enter international markets, there will be no chance of winning international markets or of greatly increasing the share of China's new-technology and high-technology products in total exports. In a certain sense, externally oriented management cadres and scientific and technical entrepreneurs are the mainstay in vitalizing China's economy and developing science and technology. We must concentrate on selecting suitable personnel from among those who have studied abroad and those with doctoral, master's and bachelor's degrees, giving them intensive training to make them into externally oriented industry managers who are bold pioneers and are capable of running operations. We should also assimilate students studying abroad and involve them in foreign-oriented enterprise activity. The salaries and benefits of marketing personnel should be linked to results, making it possible to approach international standards. We must enhance the social standing of high-technology entrepreneurs, and their income should be linked to development results. Entrepreneurs have high standing in the developed countries, and some entrepreneurs have even higher salaries and perquisites than presidents. For example, Lee Iaccoca, head of a U.S. company, makes a million dollars a year, more than 10 times what President Reagan makes. Reagan gave him a special honor, inviting him to serve as chairman of the Statue of Liberty restoration committee. We are planning to select annually a group of scientific and technical entrepreneurs who have had eminent achievements and increase their social standing.

E. Promote reform of the foreign trade system and external-affairs activity in order to lay the groundwork for the development of foreign-oriented enterprises. We must simplify passport handling and other procedures for leaving the country in order to facilitate departures and returns. Explicit preference policies must be developed with regard to foreign trade export powers and retention of part of foreign exchange earnings. The Beijing People's Government has already taken the lead by drafting the Regulations on the Beijing Experimental New-Technology Zone, which have already been approved by the State Council; Beijing has made a major stride in this area and has embarked on an epoch-making experiment.

IV. Successful Experience and Successful Paths

Hatching a group of foreign-market-oriented enterprises spearheaded by research and development institutes, creating scientific and technical enterprise groups, developing foreign-oriented industries, increasing the share of new-technology and high-technology products in our exports, and winning international markets constitute an essential method of vitalizing the economy and developing science and technology. Pace-setters both abroad and in China have furnished successful experience in successfully "making the crossing."

In 1984, the Summary Report on Reform of the Chinese Academy of Sciences which the CAS submitted to the party Central Committee and the State Council, suggested that some development activities should be organized in the form of companies. As reform, opening up and invigoration proceeded, the Academy of Sciences felt that individual organizations were not sufficiently strong to compete singly with foreign technology and foreign manufacturers and merchants and that it was therefore necessary to break down the old system and to unite; it therefore suggested a new approach to developing China's high-technology industries. As a result of several years' effort, the CAS has already established more than 300 high-technology companies. Several academic-committee members personally took the lead in a resolute battle for markets. At their instance, 6,000 to 7,000 personnel transferred from institutes to companies. Optics expert Comrade Wang Daheng [3769 1129 3801] took the lead in organizing the Daheng Company, which expresses the belief that we must not let China's optics capabilities degenerate in our hands. The Sanhuan Company's neodymium-iron-boron permanent magnetic material has a magnetic field intensity more than 100 times as strong as that of earlier magnetic materials, and one small piece of it can replace several dozen kilograms of ordinary material; organizations in the United States and Japan are now eager to make business agreements. The BGO [bismuth-germanium oxide] material produced by the Shanghai Silicate Research Institute is extremely effective in high-energy particle detection and measurement. The institute has recently received orders worth $8 million and has captured the international market. Plans are being made to establish a plant in Singapore to produce a micropore calcium silicate humidifying material developed by the institute for sale on world markets. The new "Vit-C" process developed by the Beijing Microbiological Institute was sold to a Swiss organization for $5.5 million.

The above examples indicate that China has many high-technology and new-technology products that can compete on world markets and capture a market share. The Chinese Academy of Sciences has already taken a stride: what reason do our industrial departments' research and development institutes and our colleges and specialized schools of science and engineering have for any further hesitation or indecision?

Ten years ago, Mr Yang Jiachi [2799 0857 1062] took me to visit Finland; I recently visited it again. Finland has undertaken 10 years of internationalization which have led to startling changes. Under the slogans "All the world is our workshop" and "All the world is our market," last year Finland's

per-capita national income exceeded $14,000. Now the world has become its kitchen. Finland has a population of under 5 million, less than half that of Beijing, and what it has done, our research institutes can do. But nobody here is doing it. The car telephones that Finland produces at $2,000 apiece have won 13 percent of the world market and are likely to win the China market as well. China's Daqing oil field has spent several million dollars to buy a full set of this equipment, including a central station. Recently the Finns also won orders from Guangdong. The Finns happily stated that the cooperation has been very good; hearing this, we could not decide whether to laugh or cry. On the one hand, we respected their market development ability, while on the other hand, we were uncomfortable: many of our research institutes could have made a product better than this middle-grade Finnish product. But our people lack a feeling for large markets, and they have let internal markets be snatched away, to say nothing of inter-national markets. Powder metallurgy is a field that Chinese scientists developed, but the Finns' powder-metallurgy products are now being sold on international markets. We are better than they in harmonic gears, but they have a full range of sizes that meet needs of a variety of users, which are already being sold in dozens of countries. They now even have a company that sells other countries' satellite maps, and last year they earned $35 million from this activity alone.

Foreign trade and export is the most profitable form of commerce. The developed countries use foreign trade to maintain high national income. Finland, with only half the population of Beijing, is among the 30-odd countries in the world that have more than 100 failing companies. The Finns state that what they are buying abroad is not actually enterprises, but marketing networks. Usually, when they buy an enterprise they establish a base to invigorate a market abroad. Sometimes, in order to sell their products in a certain country, they boldly hire people from that country to open up markets. One company had only 400 native personnel and 1,000 foreign-born personnel. This approach of using every possible measure to go after world markets merits our serious consideration.

China's reforms have entered the critical stage. The scientific and techni-cal sphere has taken on the glorious mission of "moving a million heroes across the river." We must effectively organize personnel to open up toward our 18,000 miles of coast and to international markets, make use of the new technology and high technology that we possess, doing our utmost for China's advancement. We must realize that only economic advancement can bring about the advancement of science and technology, education, and the entire people.

Prerequisites for Breakthrough in Software Industry Outlined

40080023b Beijing ZHONGGUO KEJI LUNTAN [FORUM ON SCIENCE AND TECHNOLOGY IN CHINA] in Chinese No 4, 1988 pp 18-20

[Article by Xu Lianfang [1776 5114 5302] and Wang Shuying [3769 2885 6601]: "Break Out Into a New Situation in the Software Industry"]

[Text]   I.   The Current Situation of China's Software Industry Is Unsatisfactory

The current situation of China's software industry is this: No breakthroughs have thus far been made, software has not established itself as a commodity, and there are practically no companies that rely on software services.   Many believe that since the current software demand on the world market is very large, since China has abundant personnel resources and many people with good mathematical talent who are good at difficult brain work, and since in addition China's labor costs are relatively low, if we developed our software industry we would have a chance of winning some international markets.   But the current situation is that in 1982-1986, the cumulative value of China's software exports did not exceed US$10 million (including more than US$2 million in data entry earnings), far below what had been hoped for.   Even though in recent years our range of computer applications has been expanding continuously and software development and service activities are becoming widespread, these types of activities are limited to in-house applications and constitute a research type of activity, and the extent to which they have been converted into projects and commodities is very poor; many products are low-level duplications.   Even in the case of the few non-in-house commissions for development and services the situation is essentially the same.   These circumstances are far from satisfactory.

It should be noted that the current status of our software industry is not at all in tune with the domestic and foreign demand situation.   The international commerce in software already exceeds $40-50 billion and is expanding at an annual rate of more than 20 percent, so that even some developing countries' software industries are expanding rapidly.   For example, India's annual software exports have already exceeded $40 million, and although Singapore has a population of only 2.8 million, with only a few thousand programmers, it has resolved to become the greatest software center of the

Far East by 1990. This places great pressure on us. Some who have investigated the history of the software industry in the developed countries have concluded that when a country's total of large, medium-size and small computers approaches 3,000, it is ready to develop a market for a software industry. China already has more than 7,000 computers in these categories, as well as more than 100,000 microcomputers, but its software industry has not yet taken shape; this unavoidably indicates the basic fact that our computer applications benefits are universally low and that the degree of commodity conversion of software is inadequate.

We must realize that the significance of developing a software industry consists not only in opening up a new industry and producing output value per se: even more importantly, software serves the development of every branch of the national economy and increases the overall benefits of the national economy. The extent of computer applications is an important measure of a country's modernization, and the extent and quality of computer applications are also directly dependent on software, so that the value-increasing effect of software in the national economy far exceeds its own intrinsic value.

The level of China's productive forces is currently not high and its commodity economy is not very well developed; these factors have a certain effect on the opening up of the software market. They preordain that China's software market cannot be as large or as active as those of the United States and other developed countries. But this by no means indicates that we lack a market. We already have 7,000 large, medium-size and small computers and more than 100,000 microcomputers, which surely constitute a market spread out before us. As China's commodity economy continues to develop, and as the range of computer applications expands, people will need more software, just as they need televisions and automobiles.

II. Developing the Software Industry Requires First and Foremost a Change in Concepts

In discussing the development of the software industry, we must first make clear the field to which its development belongs: Does it belong to the field of computer science, or to economics? The answer is clear: The development of the software industry belongs to the field of economics. But in practice, this fact is not clearly understood, or is inadequately understood. This is indicated by the fact that in discussions of the development of the software industry, the focus is usually on its technological aspects, and many opinions and suggestions lack support from economic theory, and some even are at odds with economic principles. If we limit ourselves to considering technical questions, do not go beyond the scientific sphere, and do not treat software development, circulation, and services as a complete economic activity, it will be very difficult to make breakthroughs in the establishment and development of a software industry.

This noneconomic view of the software industry has historical roots, but it is also related to the distinctive characteristics of software itself and to real social circumstances. Because China's earliest software development sprang

from the university and research organizations, software development was always treated as a research activity of such organizations. As a result, whenever its development was discussed, it was always by consultants who were computer experts, and very few economists were involved. In addition, in an environment in which the commodity economy was not well developed, the non-material character of software made it easy for people not to regard it as a commodity. These types of conceptions and actions hindered the establishment of an economic view of the software industry. Even if discussions of software development sometimes touched on the economic development of the industry, owing to lack of clarity, in most cases it was not seriously or comprehensively considered. Of course, the development of the software industry involves several important links of economic activity, and if one of these links is missing, the entire industry will have a difficult time developing. A complete market mechanism and an entrepreneur environment are the key to dealing with the weak link, but China currently lacks such a macroscopic environment. If we do not take the necessary steps, a natural solution of some of the currently existing problems will be impossible. The development of China's software industry includes an establishment stage, during which the appropriate measurements must be taken. Countries other than the United States have relied on their specific circumstances in developing the software industry and have drafted appropriate policies. But no matter what measures are taken, the first problem is to understand clearly the nature of the problem. For China, the conception of software industry development as a scientific research activity must be replaced by the view of it as an economic activity when considering measures to be taken if these measures are to be effective.

III. The Critical Factor in Developing the Software Industry

As in the case of other industries, the software industry's component elements must include scientific research, production, marketing and service. These links both constrain and promote each other and are organically combined into a single whole. If there is only a research system but no production system, or if there is only software development but no marketing and services, then it is very difficult for a beneficial cycle to develop in the software industry. But the current state of affairs in the software industry is that the production, marketing and service links are seriously out of contact with each other, and the majority of software development organizations stop with research activities or take a stand-offish attitude toward the users, while pure software enterprises are very few and have difficulty surviving; this is the crucial factor limiting rapid development of the software industry.

The difference between the software industry and other industries is that in other industries the line of demarcation between research and production is very clear, but this is not the case in the software industry. Because software production is nonmaterial and nonrepetitive, in many fields, and even in different parts of a single field, software production has a certain "creative" character: it is not repetitive production, so that the line of demarcation between the research and production fields in the software industry is not as clear as elsewhere. Precisely because software

development is creative, nonrepetitive labor and generally yields results, advanced schools and research organizations all want to engage in it. But it should also be pointed out that advanced schools and research organizations are institutions [rather than enterprises], and when their research activity produces results or products this is not the same as converting them to commodities. And because software is not converted into commodities, its utility value is very hard to embody. Currently, advanced schools and research organizations have considerable software production capabilities and are in the advantageous situation of not having to consider production costs, while pure software production enterprises face a serious shortage of software production capabilities and unequal competition for the software market, as a result of which it is hard to start up such pure software enterprises and keep them in existence. This is one side of the question. The other side is that because research-type software development is somewhat removed from the user's real requirements, users increasingly feel that entrusting them with software development will not satisfy their needs, and as a result they are tending to develop software themselves (i.e., so-called "in-house development"). They have no choice but to develop it themselves, and they do it with inadequate personnel and technology. As a result, not only is the quality of their software development rather low, but it is not in accord with the principle of specialized division of labor and the result is to further weaken the software market, which is difficult to develop in the first place, and it thus becomes a further hindrance to the development of the software industry.

Nonproductive software development cannot stimulate demand, and inadequate demand limits production; nonproductive software development results in large amounts of waste and losses; and nonproductive software development can only have the consequence of making the software industry hesitant and unable to advance beyond the research stage. We should therefore rectify this state of affairs as quickly as possible. We hope that software will be exportable and will capture certain international software markets. Similarly, foreigners also hope to export their software and to capture our markets. If our software industry cannot develop, we will not only be unable to move out into the world, but we may also lose our domestic software market. The fierce competition in the international software market cannot be changed by anyone's subjective wish. If we should lose our own software market, it will be difficult to win back again because powerful economic and social momentum is very hard to reverse.

IV. Measures To Break Out of the Current Situation

There are specific measures that can be taken in order to break out of the present situation. We suggest that measures be taken in the following three areas.

A. Establish cognizant management organs for the software industry.

It is very hard to imagine comprehensive establishment and development of an industry without an industry management organization. The establishment and development of the software industry involves a wide range of factors such as

regulations, standards, policies, norms, legislation, management, publicity, and cadre development. But the most important thing is to have a cognizant organization to take responsibility for this work; otherwise it will be difficult to make any of the work run smoothly. The responsibility of a cognizant management organization for the software industry would consist of: carrying out software industry policy, strengthening the software industry's macro-scale management, and promoting the comprehensive establishment and development of the software industry.

B. Start up joint-capital software enterprises.

Establishing joint-capital software enterprises will make it possible to use foreign funds and market channels and to absorb foreign management experience and software development technology; it will also become possible to use abundant foreign capital to compensate for our insufficiency of funds and to accelerate the use of our advantages in personnel and brainpower resources, which will give an impetus to the development of China's self-capitalized software industry and make our software industry take off from a rather high starting point.

C. Create conditions favoring the survival of the software industry.

For the software industry to come into being and develop normally, we must start by taking flexible "one-step-forward, one-step-back" measures. The "step forward" involves encouraging traditional industrial departments or units to invest in the software industry. In addition, the software industry must rely on the traditional industries for its survival and development. There are many examples of this survival and development model. For example, in the United States, IBM developed as a computer hardware company, and AT&T originally was the Bell Telephone Company, a major computer user. The desirable aspects of this model are that it does not require large state investment, but relies on the traditional industrial departments or units for investments and venture capital. The software industry is not necessarily independent of and separate from the traditional industries, but it must consist of true enterprises. The "step back" aspect is that we must limit productive software development activity by advanced schools and research organizations. They should engage in research and break new ground while leaving productive software development to the software enterprises. They may of course cooperate with software enterprises. The advanced schools and research organizations are a major technical force in the development of China's software industry, and we must encourage them to cooperate with the enterprises or to convert themselves into enterprises and involve themselves in the economic activity of the software industry. Standards should be developed for the software research activity of advanced schools and research organizations, and software science should be strictly distinguished from production in order to guarantee that research activity does not replace productive activity.

In addition, we must create a market mechanism for the development of software enterprises. Software enterprises must have a market if they are to survive. At present, a software market system is far from taking shape in

China, and the software enterprises are few and weak; as a result, there is a great need for the state to provide as much support as it can in the market area. State software development projects must be used as a means of supporting the survival and development of software enterprises and the development of a national software market. For example, many large state computer applications systems have relatively abundant funds, and they are an important component of China's software demand. It is quite permissible to alter the previous distribution method, in which the tasks were handed down from above, and to use limited but still comparatively abundant capital to support the survival and development of software enterprises. Using projects to support enterprises produces better results than direct investment in enterprises, since it assures completion of state projects and promotes the development of the software industry; it is therefore an important way of producing a breakthrough in the software industry.

Development of Export-Oriented High-Technology Industries Urged

40080023c Beijing ZHONGGUO KEJI LUNTAN [FORUM ON SCIENCE AND TECHNOLOGY IN CHINA] in Chinese No 4, 1988 pp 35-36

[Article by Ma Xiguan [7456 6932 0385]: "Support the Development of Foreign-Oriented High-Technology and New-Technology Industries"]

[Text] Among the developing countries, China has rather strong scientific and technical capabilities. But as a result of policy and organizational factors, our scientific and technical advantages have not been thoroughly utilized, and the potential for using science and technology to promote accelerated economic development has not been realized. In order to turn this situation around, we must act in two areas: first, we must implement the contract management responsibility system in order to strengthen the competitive mechanism and promote enterprise technical development; second, we must promote the conversion of scientific and technical results into production capabilities so that science and technology more effectively support production and export. We must mobilize and organize our scientific and technical personnel, involve them in international competition and use certain of their scientific and technical results to develop foreign-oriented high-technology and new-technology industries and to experimental development zones, produce exportable products that can earn foreign exchange, and develop a foreign-oriented economy.

Overall, the experience of the developed countries in developing high technology and new technology include four main aspects: 1) They have abundant scientific and technical resources, personnel resources and available scientific and technical results; 2) the governments use a preferential support policy; 3) they have sufficient capital sources; 4) they select good geographic environments.

Although we are behind the developed countries economically and in terms of science and technology, China currently has about 8.5 million scientific and technical personnel, including about 370,000 research personnel. If we could use a special policy and adjust our operations system, it would be possible to mobilize a group of scientific and technical personnel and involve them in heading high-technology and new-technology enterprises. As regards the stock of scientific and technical results, we have already achieved more than 60,000 major results, of which about 16,000 are at the ministry or province

level. It would be entirely possible every year to identify among these results a group of projects suitable for export and to organize their development and production. If effective measures were taken as regards fund raising and support policies, China's high-technology and new-technology industries and their experimental development zones would be able to make progress.

When developing industries of this type, other countries have had four main sources of funding: 1) government funds; 2) enterprise investments; 3) bank loans; 4) venture capital. Drawing on foreign experience, when China has developed high-technology and new-technology industries, particularly in the coastal areas, it should benefit fully from local advantages, making use of a variety of methods such as bank loans, venture capital, stock issues, enterprise, research and advanced school fund raising, and fund raising among the people to obtain funds. These funds should be used for targeted support of important links in the conversion of scientific and technical results into productive capacities, and for funding special-project efforts, demonstration projects, dissemination and enterprise development and expansion. As regards the investment mechanism, we should use a combination of soliciting of bids, the cooperative system, compensated utilization, distributed investment, and support for the best.

In the process of developing high-technology and new-technology industries and experimental development zones, we must proceed, in accordance with China's circumstances, to make use of the socialist commodity economy mechanism and put scientific and technical results capable of earning foreign exchange into production; on the other hand, we must make use of the regulating effect of the market mechanism to encourage many parties to run a variety of types of high-technology and new-technology enterprises. Examples are: running export industry showrooms and offices in the developed countries; running domestic enterprises centered on development institutes and advanced schools; enterprises run by the people; and encouraging the technical personnel of research institutes and advanced schools to run integrated technical-industrial-sales organizations engaging in development, production and marketing.

In order to encourage the development of China's high-technology and new-technology enterprises and experimental development zones, we suggest that the following nine effective support policies and measures be used.

1. Taxation

a. Exemption from income taxes for all new high-technology and new-technology enterprises for 3 years, followed by collection of only 50 percent of the tax for the next 3 years.

b. After this period of exemption from or reduction of income taxes elapses, linkage of the tax rate to the percentage of output that is exported. For every 10-percent increase in the percentage of an enterprise's total output value that is marketed abroad, the tax rate should be decreased by 5 percent, with the minimum tax rate being 15 percent (as in special economic zones).

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c. Permit such enterprises to treat fixed capital investments used in scientific and technical development such as purchases of scientific instruments and equipment as enterprise costs.

d. Exemption of these enterprises from the tax on medium-scale trial production products and the construction tax.

e. When enterprises need to import instruments and equipment that cannot be produced domestically for the purpose of scientific and technical development, these instruments and equipment should be exempt from the import tax for 5 years.

All of the above tax decreases or exemptions should be treated as "state support funds," and the enterprise should be able to use them specially for new-technology development and production development.

2. Export-Related Management Rights

With permission, it should be possible to establish integrated import-export companies in the high-technology and new-technology development zones and high-technology and new-technology enterprises with rather large-scale import and export business; these should be given foreign trade and import-export powers. With permission, these enterprises should be able to set up technical, economic and trade organizations abroad, and hold talks, trade fairs and similar foreign-oriented technical, economic and trade activities.

3. Product Prices

Enterprises should be able to set their own prices for new high-technology and new-technology products that they have developed and for products for which the state has not set uniform prices.

4. Retention of a Percentage of Foreign Exchange Earnings

All of the foreign exchange earnings of enterprises of this type should be retainable by the enterprises for their own use for 3 years; in the next 3 years a 20-80 plan should be in effect, with the enterprises retaining 80 percent of earnings and the state and locality receiving 20 percent. The enterprises should be allowed to dispose of these retained funds as they see fit. Enterprises of this type should be allowed to establish foreign-exchange accounts in the Bank of China and withdraw them and use them as they wish.

5. Simplified Procedures for Travel Abroad

In the case of enterprises involved in import or export of high technology or new technology, quotas should be drafted and universal commercial departure passports should be issued; the approval procedures for travel abroad should be simplified. On the first occasion that technical and commercial personnel go abroad, the government should issue and approve the visa, but if they go abroad more than once within a year the enterprises should have the right to give approval.

6. Encouraging Personnel Mobility

In order to encourage scientific and technical personnel who wish to work for high-technology and new-technology enterprises, we should implement a two-way selective personnel mobility system. We should implement the appointment system, the contracting system and the visiting staff member system. Scientific and technical personnel should be permitted to take temporary transfer, resign, retain the original post without salary, or moonlight in order to create, head, contract for or lease all types of high-technology or new-technology enterprises. Enterprises should be permitted to directly recruit university graduates, graduate students, students studying abroad and scientific and technical personnel who are abroad; the enterprises must guarantee their legal rights and income.

7. Personnel Matters

Scientific and technical personnel or technical workers who are transferred to new-technology or high-technology enterprises retain their standing as cadres or workers under the system of ownership by the whole people; their seniority continues to accumulate, the work-file wage method is used while they hold the position, and they maintain their pensions. Wages are decided by the enterprises.

8. Technical Ranks

In accordance with the contribution and technical level of scientific and technical personnel, and with reference to state specialized scientific and technical job evaluation standards, high-technology and new-technology enterprises run by the people should be able to take it upon themselves to appoint scientific and technical personnel to specialized technical posts.

9. High-Technology and New-Technology Development Parks and Zones

In addition to enjoying the above eight preferential policies, when feasible, high-technology and new-technology development parks and zones that have received permission should enjoy the following benefits.

a. High-technology and new-technology enterprises in development zones should be exempt from income taxes during the first 3 years of their existence; in the 4th through 6th years, the income tax rate should be 7.5 percent, and starting in the 7th year the rate should be 15 percent.

b. When the output value of exported products and products replacing imports produced by enterprises in the parks or zones exceed 40 percent of the enterprises' total output value, the income tax rate should be 10 percent.

c. Land use by enterprises in the parks and zones should be given preference comparable to that of special economic zones.

d. Accelerated depreciation should be applicable to instruments and equipment used by enterprises within the parks and zones for development of new technologies and new-technology products; the depreciation period should be shortened to 4-7 years (it is currently 12 to 20 years for ordinary enterprises).

Suggestions on Building Up Biotechnology Industries

40080023d Beijing ZHONGGUO KEJI LUNTAN [FORUM ON SCIENCE AND TECHNOLOGY IN CHINA] in Chinese No 4, 1988 pp 41-43

[Article by Weng Yannian [5040 1693 1628]: "Some Suggestions on Building Up New Biotechnology Industries in China"]

[Text] It has been no more than 15 years since recombinant DNA technology appeared. During this brief period a new science and technology, known as biotechnology, has been developing rapidly throughout the world, and companies and enterprises engaged in developing biotechnology products have been springing up everywhere in the developed countries, particularly in the United States. These facts announce to the world that man will be able to create species and materials that the natural world has never produced, that man will break out of the traditional forms of production and use entirely new forms and achieve unprecedented efficiency in producing large amounts of the necessities of life, and that in the next century man will overcome such major diseases threatening human health as tumors, genetic diseases, cardiovascular disease and viral diseases (including AIDS). In sum, we may predict that in the next few decades, biotechnology, microelectronic information technology and new materials will be the three main pillars of the world revolution in new technology. The biotechnology industry will become one of the most promising industries of the next century, and it will have a decisive position in the national economy and in social development.

Traditional biotechnology has a very long history. The ancient soy-sauce and fermenting and brewing techniques and more recent MSG and antibiotic production are representative examples. But the modern biotechnology to which we refer is a high technology based on gene recombination and modification, cell fusion and hybridoma technology, plant tissue culturing and test-tube breeding technology, large-scale culturing of plant and animal cells, immobilized enzyme and cell technology, and new biological reactors and product isolation and purification technology. Industries based on these high technologies are commonly caused high-technology biotechnology industries.

A characteristic that the biotechnology industry shares with other high-technology industries is that they are all knowledge-intensive, technology-intensive and funds-intensive. In addition, the biotechnology industry has

its own distinctive characteristics, based on the fact that bioresources are reproducible and that its sources of raw materials are inexhaustible. Because biological reactions generally proceed at constant temperature and pressure, it uses microbial fermentation processes and biochemical processes as a substitute for chemical processes, with the result that the biotechnology industries also have low energy consumption and produce no pollution.

I. Development of Chinese and Foreign Biotechnology and Biotechnology Industries

Internationally, biotechnology has already been developing for 15 years and has had encouraging achievements in medicine, light industry, foodstuffs and agriculture. Man-made insulin, man-made growth hormones, hepatitis B vaccine, interferon, a vaccine for infant diarrhea of livestock, and other products produced by genetic engineering have already gone on the market, and several dozen additional genetic engineering products are under development; a group of monoclonal antibody diagnosis kits and clinical diagnosis enzyme kits are already in widespread clinical use; plant tissue culturing and rapid breeding technology are already in wide use in flower, plant and fruit tree production; progress in the use of plant genetic engineering to improve higher plant characteristics has been faster than expected; and protein engineering, which has been hailed as second-generation genetic engineering, is already beginning to show its strength.

The United States is taking the lead worldwide in establishing new biotechnology industries. A large contingent of U.S. professors and scientists have left the laboratories and have joined with engineering and technical personnel to establish several hundred venture companies for the development of bioengineering products, thus making a contribution to the creation of new biotechnology industries that have attracted world attention. Many of the first group of genetic engineering products to go on the market, such as those described above, were developed by U.S. companies. Currently there are more than 700 biotechnology companies in the United States, with a total capital of $15 billion, and the biotechnology industry has already attained a considerable size. Japan made a slightly later start in biotechnology and it lags behind the United States in basic research. But as a result of its great strength fermentation engineering, it is superior to the United States in downstream technologies and in development capabilities. Its approach is to bring together the governmental, academic and commercial spheres in a bid to surpass the United States, and its industries are developing rather rapidly. Several of the main European countries, such as the FRG, France, and England, have vigorously drafted biotechnology development policies in an attempt to match the United States and Japan. Their approach is one of gradually increasing investments, international integration, and coordinated development.

The biotechnology industries have attractive development prospects. A U.S. technology development center forecasts that the market scale of world biotechnology products will be $27 billion in 1990 and that in the year 2000 the market in the United States and several European countries will reach $160 billion. Relevant departments in Japan predict that by the year 2000,

the market scale of Japan's modern biotechnology products will reach $62.5 billion. Even though predictions by specialists in the various countries regarding the mid- and long-term market scale of biotechnology are not entirely in agreement, it is indisputable that the future biotechnology market will be huge and will continue to expand.

In China, biotechnology research began in the late 1970's, not too far behind other countries. As a result of the efforts of a multitude of scientific and technical personnel, in slightly more than a decade it has achieved some gratifying research results. Examples are the use of genetic engineering to develop a hepatitis B vaccine, interferon, a diarrhea vaccine for young pigs, a genetically engineered microorganism producing penicillin acylase [as published] and plants engineered for resistance to cucumber mosaic virus and herbicides; of 14 varieties of protoplasm-reproduced rice, corn and soybeans, 8 are world firsts; hybridoma technology has been used to develop a group of medical and agricultural monoclonal antibodies; the new generation of enzyme preparations developed by immobilized enzyme and cell technology are also being used in clinical diagnosis, pharmaceutical production and foodstuffs production; and many research results have also been obtained in plant and animal cell engineering, breeding, and plant tissue cultivation and rapid reproduction technologies. Some of these results are already in small- and medium-scale trial production, and some are already in widespread production. But it should be noted that, as a result of the weakness of China's basic research in biotechnology, we are lagging somewhat behind in downstream technologies, so that overall, Chinese biotechnology is in the imitation and catch-up stage; there are very few path-breaking developments, and our overall research level is about 5 to 10 years behind the world leaders. Even though in recent years a few biotechnology products have entered the market, we are still in the preparatory stage as regards the conditions for development of a high-technology industry. Thus, the large-scale development of China's high-technology biotechnology industry will come in the teens or twenties of the next century.

II. Some Suggestions for Developing China's Biotechnology Industry

A. Strengthening basic research in biotechnology and downstream technologies is the key to promoting biotechnology in China and its development as an industry. Considering the overall history of biotechnology in the developed countries, we see that there are two reasons for its rapid development: First, it was backed up by solid basic research; and second, it was supported by advanced biochemical engineering technologies, so that laboratory research results were converted rapidly into products. But these two areas are China's weak points. As a result, for biotechnology to develop rapidly in China, we must make a strong effort on basic and key technologies and on downstream technologies, focused chiefly on biochemical engineering. Effective work on these two crucial factors is essential if biotechnology research and development is to be accelerated.

B. Objectives and Pace of China's Establishment of Biotechnology Industries. Because China's research and development level in biotechnology is low, it is estimated that even with a vigorous effort, we are in a position to establish

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new industries before the year 2000 only in the fields of diagnostic mono-clonal antibodies, immobilized enzyme preparations (clinical diagnosis enzyme kits and immobilized enzymes and cells for use in drug production, light industry and foodstuffs production), plant tissue culturing and rapid breeding. As regards genetic engineering, it is estimated that by the end of the century only a few products can be thoroughly developed, and an industry of respectable size is unlikely to have taken shape. In view of constraints on the technical capabilities, funding and industrial support needed by biotechnology, before the year 2000 our only course is selective development of the three rather mature technology areas described above as limited objectives; this will be done in several steps.

The first step is to select ten-odd products that currently or soon can go into medium-scale experimental production as development projects and estab-lish ten-odd modern biotechnology plants as sources of experience and demon-stration projects for future development of new industries.

As the second step, we should strive to establish biotechnology industries by the year 2000 in three fields: medical and agricultural diagnostic mono-clonal antibody kits, immobilized enzyme preparations (including clinical diagnostic enzyme kits), and plant tissue breeding and rapid reproduction. With these products, together with those in other fields of biotechnology, it is estimated that by the end of the century China's high-technology biotechnology industry can achieve an output value of about 15 billion yuan.

In the third step, although in the first half of the 21st century biotech-nological development may well have resulted in certain unimaginable new fields and areas of growth, China's development in biotechnology will not yet have brought it to the forefront. China will have made great progress in genetic engineering, especially protein engineering; in monoclonal and targeted drugs for use in treatment of humans; and in plant genetic engineer-ing. It is estimated that high-technology industries in these fields will not take shape before the twenties decade of the next century.

C. A Model for China's Development of Biotechnology Industry. There are numerous foreign models for the development of biotechnology industries. The United States used specialized private venture companies to develop entirely new biotechnology products. Many hundreds of biotechnology com-panies were established in Washington state's "Bioengineering Valley," in "Gene Valley" near San Francisco, and on Route 128 around Boston; they took the lead as biotechnology production bases that attracted world attention. The Japanese took a different approach: they implemented a trilateral development model involving private enterprise, state research organizations, and advanced academies and schools. Their development structure was based on existing enterprises and made use of the powerful existing fermentation engineering industry, gradually forming a new biotechnology industry. We are far below the United States in terms of our level of basic research and venture capital capability, and we are no match for Japan in terms of down-stream development technologies and capabilities, and thus in developing biotechnology industries we can imitate neither the U.S. nor the Japanese model. To develop high-technology industries requires technologies and

funds: these are two essential conditions. Under China's circumstances, in developing the biotechnology industry we should adopt the following model: have scientific research units or advanced schools take the lead, unite research units, advanced schools with enterprises that have relatively advanced technological capabilities and facilities, combining the strong points of each type of organization, to create an integrated development company or combine. In addition to some venture capital from the state, multichannel fund-raising should be carried on. This is the principal model for developing China's biotechnology industries before the year 2000. As regards entirely new biotechnology enterprises, our only recourse is to operate a small number of experimental enterprises, gain experience, and proceed gradually.

D. Intensified training of technical personnel in biotechnology engineering is an urgent task. Because biotechnology is a popular science worldwide, many Chinese students and persons doing advanced work are studying abroad or participating in biotechnology research. A research contingent has also been developed domestically through research and practical activities, so that there is no lack of talent. But very few Chinese are studying biochemical engineering (the downstream technology) abroad; this is primarily because for reasons of commercial competition, foreign biotechnology companies are not willing to let foreigners take part in their development work. In addition, for a relatively long period, insufficient importance was attached to training biochemical engineering personnel in this country, which has led to the current shortage of personnel in the field. This shortage has already become a constraint on China's development of biotechnology industries. For the next few years, a high priority should be attached to a variety of vigorous measures to strengthen the training of technical personnel in biochemical engineering.

E. Modernizing traditional biotechnology industries by means of modern biotechnical research is an activity of strategic importance, and we should draft an appropriate technological policy. It is estimated that of the total output of China's biotechnology industry, traditional biotechnology will account for 80 percent in 1990 and still for about 70 percent in the year 2000. This makes it clear that using modern biotechnology and its research results to modernize the traditional biotechnology industries is an extremely necessary and arduous task. There are two main approaches to modernization: One is to use genetic engineering and cell engineering technology to create new varieties of engineered microorganisms to replace microorganisms bred by conventional methods (such as varieties producing amino acids and enzymes), and to greatly increase output; the other approach is to replace traditional techniques and processes with the new techniques and processes of modern biotechnology: for example, use of immobilized enzymes and cells to replace traditional enzyme preparations; the use of new biological reactors and new separation and extraction technologies to replace old reactors and outmoded isolation and purification techniques. The advantage of this method is that the investment required is small and the return is rapid, so that a considerable group of traditional biotechnology industries can be modernized rapidly.

Torch Plan Moves Forward in Jilin

40080036a Changchun JILIN RIBAO in Chinese 22 Aug 88 p 1

[Article: "High-Technology Research Indirect Benefits Approach 100 Million Yuan"]

[Text] On 18 August, the [Jilin] province science and technology committee assembled the heads of science and technology committees from all localities, cities and prefectures in the province, the officials of some colleges, specialized schools and research institutes, and the research offices of some large enterprises to convey to them the spirit of the first national Torch Plan conference. The participants conscientiously investigated the significance of developing high technology and new technology in Jilin Province and made suggestions on ways of implementing the Torch Plan.

The Torch Plan is a plan for developing high-technology and new-technology industries and is one of the mainstays of the modern economy. Recently the party Central Committee and the State Council authorized its nationwide implementation.

In the last few years, the province has arranged a total of 204 high-technology research projects and has obtained 80 high-technology results. A group of combined optical-mechanical-electrical high-technology products has been developed, a series of applied computer software has been developed, and independently developed new varieties of materials have been appearing in increasing numbers. More than 20 enterprises in the province are producing high-technology products, with an annual output value of over 50 million yuan, yielding an indirect economic effect of nearly 100 million yuan. These circumstances provide favorable conditions for implementation of the Torch Plan in Jilin Province.

An official of the province science and technology committee who spoke at the meeting said that at present insufficient funds are being invested in high-technology research in the province, which is seriously hindering its development. High technology is a high-investment, high-output-value, high-effect undertaking, and larger funds allocations are needed to support it. The province science and technology committee is planning to establish a Torch Plan development fund and will take all possible steps to give support and encouragement to large academies and institutes as they help promote the development of high technology in the province.

State Official Discusses Contract-Related S&T Achievements, Rights

40080030a Tianjin JISHU SHICHANG BAO [TECHNOLOGY MARKET NEWS] in Chinese
24 Aug 88 pp 1, 4

[Article: "State Science and Technology Official Discusses Evaluation of
Scientific and Technical Results and Rights Questions Related to Imple-
mentation of Science and Technology Contracts"]

[Text]   Editor's Note. The "PRC Technology Contracting Law" was
issued on 1 November 1987. With State Council authoriza-
tion, the State Science and Technology Commission issued
the Temporary Technology Contract Management Regulations
on 21 March 1988. China's technology market has entered
the legal sphere and the general situation is good. But
the conversion of technological results to commodities is
a new undertaking, and some departments and localities
report that they have encountered certain conflicts
regarding technology rights and evaluation; they feel
that the legal provisions are rather general, and the
details of implementation have not yet been made public,
so that they are having difficulty in dealing with these
situations, and they would like the specific limitations
of the policy limitations to be explained. As a result,
the State Science and Technology Commission offers the
following clarifications on some widely occurring problems.
After the Detailed Principles for Implementation of the
Technology Law are issued, if any clarifications are not
fully in agreement with them, the Detailed Principles will
be taken as the standard.

[Question] 1. What standards are to be used regarding acceptance of
technological results that have been produced under technology contracts?
How are the parties to deal with conflicts regarding technology results
evaluation?

[Answer] Technological contracts are legal forms by which the parties
carry out technological development, technology transfer, technological
consultation and technical services; the contents of the contracts and the
technological results to which they apply are diverse and rapidly changing,
and it is difficult to give uniform acceptance standards or to carry out

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acceptance in terms of such concepts as "advance" or "mature." Consequently, the State Technology Contracting Law specifies that acceptance standards and methods for technology contracts must be agreed upon by the parties. In other words, whether technical results resulting from technological contracting are up to standard should be decided in terms of technical characteristics, economic benefits or other requirements agreed upon by the parties, and evaluation must be made by a procedure agreed upon by the parties. In cases where the contract specifies no agreed-upon acceptance standards or procedures, acceptance testing must be performed in accordance with the general applications requirements.

Disputes between the parties in regard to evaluation of technological results are frequent in technological contracting. When the cognizant departments, arbitration organizations or legal organizations deal with disputes of this type, they must adopt a realistic, scientific attitude, hear opinions from the relevant scientific and technical departments and experts, and make a fair evaluation of the results. Where necessary they can organize a group of experts to make an evaluation in terms of whether the results is in accord with the terms of the contract or with general applications requirements.

[Question] 2. What state regulations regarding the evaluation of technological results exist? When dealing with cases involving the evaluation of technological results, can the final evaluation be made by a single expert?

[Answer] Science and technology results review is a system for evaluating technological results. In April 1961 the State Council published the Temporary Regulations on Technological Evaluation of New Products and Processes, which specified the scope, methods and effects of evaluation. Many of its provisions have ceased to be appropriate to the requirements of the current development of a socialist commodity economy, and as a result, on 3 January 1987 the State Council issued a notice abolishing those administrative regulations and stipulating that they were to be replaced by the Scientific and Technical Research Results Management Regulations issued by the State Council on 22 February 1984. In view of the fact that its provisions regarding the evaluation of scientific and technical results are rather general, and in order to make the evaluation system able to function effectively, on 26 October 1987 the State Council issued the Procedures for Evaluation of Scientific and Technological Results. These are the state regulations currently in force regarding the evaluation of scientific and technological results. Thus, if an evaluation of scientific and technical results resulting from technological contracts becomes necessary, including needs for evaluation in cases in which arbitration organizations or legal organizations are dealing with disputes over technological evaluation, the provisions of the Regulations on Management of Scientific and Technological Research Results and the Procedures for Evaluating Scientific and Technological Results, must be followed.

Results evaluation is a complex scientific and technological evaluation activity. Some problems involving long-standing expert disagreements should be dealt with by analytical methods, discussion, and democratic methods, permitting both parties the right of reply, so that the conclusions will stand the test of history. It is not appropriate simply to resort to the

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evaluation methods of ordinary civil or criminal cases, in which some individual evaluator's view regarding some technology is greeted as an "evaluation result" of evidentiary value. This is not scientific analysis, and it can readily lead to errors of one kind or another.

[Question] 3. If technological results are not in accord with the terms of a technological contract or if it is confirmed that there are major deficiencies, is the party that provided the technological results in violation of the law? Are these technological contracts invalid?

[Answer] Cases in which scientific and technical results or technical services that are covered by technological contracts are deficient or do not meet contract specifications are frequent types of breach of contract. The party providing the technology must be held liable for breach of contract and must pay a breach of contract penalty to the other party or compensate his loss. In general, if a party has not performed his duties as specified by the contract, this is not a crime, and it should not be made a basis for declaring the technology contract invalid.

The term "invalid technological contract" refers to cases in which one of the causes specified in Article 21 of the law on technological contracts exists and in which the contract is a forbidden contract by virtue of involving activities forbidden by laws and regulations. In recent years, relevant policy documents have emphasized that technology entering the technology market must be as mature, advanced and applicable as possible. Article 39 of the technological contract law specifies that the party transferring nonpatented technology transfer contracts must "assure the applicability and reliability of technology." To require legally and in terms of policy that the parties carry out this responsibility is necessary, but the question of whether a party has or has not carried out the contract obligations must not be confused with whether the contract has legal force. Similarly, cases in which it is claimed that the technological results are not mature or have not passes evaluation as a grounds for erroneously declaring legally entered-into technological contracts invalid and in which the technology provider is treated as a profiteer or cheat must be rectified.

[Question] 4. What is the meaning of "technological rights and interests? What responsibility attaches to infringing on others' technological rights and interests?

[Answer] Technological rights and interests are the totality of the spiritual or economic rights that the party legally enjoys regarding technological results. Spiritual rights are rights that are inalienable from the technology creator's person and creative effort, i.e., they are rights of status and honor of the person who produces the technological results. These rights are exclusively those of the creator of the technology and may not be infringed upon or elienated by others; in addition, they may not be altered or transferred by contract provisions. Economic rights are the rights of using or transferring the material benefits obtained from the technological result, including patent rights, patent implementation rights and the rights of use and transfer of nonpatented technological results. As a consequence, the Temporary Regulations Regarding Technical Contract

Management and the relevant regulations and policy documents refer to "infringement of others' technological rights and interests," which include three types of infringement, namely infringement of the rights of status and honor of the party who created the technical result, infringement of patent rights, patent implementation rights and patent application rights, and infringement of the rights of use and transfer of nonpatented technological results. According to the laws currently in force, technological rights and interests constitute intellectual property rights, and in serious cases of patent infringement, the persons directly responsible must be held liable in accordance with Article 127 of the criminal code. Other infringements involve primarily civil liability. The person suffering infringement has the right to request its cessation, mitigation of its effects, and compensation for loss. If one party to a technological contract infringes the other party's rights and interests, this usually constitutes infringement and breach of contract. In addition to being held liable for breach of contract, he must also be held liable for infringement. Losses resulting from infringement of technological rights and interests usually are not expressed directly as a decrease in or damage to material property, and as a result the usual methods of loss calculation in economic contracts cannot be undiscriminately applied. As a result, the technological contract law specifies that parties can agree on a method of computing compensation for losses. In cases where there are no such provisions in the contract, the loss can be inferred to be the actual benefit obtained by the infringer during the period of infringement, or the actual loss suffered by the infringee during the infringement.

[Question] 5. How are we to understand the rights of use and transfer of nonpatented technology results? Are they a kind of "ownership system" of nonpatented technological results?

[Answer] The rights of ownership and transfer of nonpatented technological results referred to in the technological contract law are rights of use and transfer of nonpatented technical results specifically between the parties, springing from legal provisions or contract terms. The right of use is the right to apply the nonpatented technological result for production or operating purposes, and the right of transfer is the right to furnish or transfer the material benefits gained from the technological result to another party via a technological agreement.

Sometimes, when units develop a technological result, they declare that they have a "right of ownership" to that result. This is not correct. A "right of ownership" is the right of a property owner to possess, use, dispose of and benefit from his property. The right of ownership is an exclusive right, and the right itself is specified, while the duty is unspecified. In the case of a technological result, only after applying for an receiving patent rights does the patent owner have the exclusive right, during the time that the patent is in force, to apply his invention or creation as in other rights of ownership. But the rights of use and transfer of nonpatented technological results are different. First, they exist only between the specified parties. Between an organization and its employee, the right of use and transfer of job-related technological results belongs to the

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organization, while the rights of use and transfer of non-job-related technological results belong to the individual who has produced them. Between the parties to the contract, in cases when it is specified that the right of use and nonpatented technological results belong to one of the parties, the other party may not use them or transfer them, while if it is specified that the rights of use and transfer belong to both parties jointly, then both parties can utilize them, but for either of the parties to transfer the result, the one party must secure the agreement of the other party, and the benefits obtained from it are to be divided reasonably between the parties. Second, it has no effect of excluding third parties. In other words, the rights regarding a nonpatented technological result are legally binding only between the organization and its employee or between the two parties to the contract and they do not affect the use or transfer of the invention or creation by any other party who has possession of the technology. In this way, the rights of use and transfer of nonpatented technologies are not exclusive rights, but are nonexclusive, and they thus do not come under the category of ownership rights in the legal sense.

[Question] 6. The technical contract law specifies that "the rights of ownership or transfer of job-related technological results belong to the employing organization," but existing policy permits scientific and technical personnel to hold spare-time jobs in order to use their technical expertise in the service of economic construction; are not the two in conflict? What is the difference between "technological results" and "technologies" as used here?

[Answer] Provided that scientific and technical personnel perform the duties of the principal job, they may use technical knowledge that they possess or have acquired in a second job in the service of economic development: this helps to make full use of the capabilities of the existing scientific and technical contingent, to promote scientific and technical advances and to develop productive capabilities. Allowing scientific and technical personnel to hold spare-time second jobs is a policy under the reform of the science and technology system and must be adhered to. It is not in conflict with the legal provision that the rights of use and transfer of job-related technological results belong to the employing organization. Technological results are products, processes, materials, or technical schemes for improving them that have been produced from existing scientific and technical knowledge. Job-related scientific and technical results are relatively complete, applicable, competitive technical schemes that were produced as a result of an employee's performance of his duties for the main employer or that primarily made use of that employer's physical and technological facilities. The right to enter intocontracts regarding technological results of this type belongs to the employing organizations, not to the individual. In addition, the individual must not take it upon himself to transfer technological results or intermediate results of research and development or key technologies which by express specification cannot be disclosed to others and for which his main employer has applied for or will apply for a patent or has applied for an award, or which the employer is planning to transfer. However,

with the exception of the above technological results, technologies which the individual possesses or acquires in his main employment, including scientific and technical knowledge, experience and information, do not constitute job-related technological results and do not fall within the employing organization's technological rights and interests, and the use of this technical knowledge in the service of economic development is not a violation of the employing organization's technological or economic rights and interests.

[Question] 7. If a contract specifies that the use of some nonpatented technological result belongs to one of the parties, can the other party use the result for new research and development?

[Answer] The right of use of nonpatented technological results is the right to apply the result for production and operating purposes. China's constitution species that the public is free to engage in scientific research. The patent law expressly provides that use of patented matters exclusively for scientific research or experimentation is not regarded as a violation of patent rights. A fortiori, the use of nonpatented technological results in research and development activity is not subject to limitation. As a result, in cases where a contract specifies that the right of use of nonpatented technological results belongs to one of the parties, the other party may not apply those results, but he can engage in new research and development based on those results and can use them in other research topics. In some contracts, one party limits the other party's use of existing technological results for new research and development by contract provisions in order to establish a technological monopoly; this type of agreement hinders scientific and technical progress and is not permitted.

[Question[8. If an employee goes on leave, retires, resigns, is discharged, is reassigned, or is retained without salary, then who gains the rights to later technological results closely related to his work for the original employer or directly belonging to his range of duties for the original employer?

[Answer] All scientific results produced in the performance of one's duties for the original employer or produced primarily by the use of the original employer's material and technical conditions constitute job-related technological results, and the right to use and transfer them belongs to the original employer. This basic principle likewise applies to the technological activity of personnel who have gone on leave, retired, resigned, been discharged or reassigned, or have been retained without salary. But technological results achieved after these actions vary in their attendant circumstances, and the situations are rather complex. In terms of the conditions under which such results were produced, there are those that made use of the original employer's material and technical facilities, those that make use of another organization's material and technical facilities, and some that make use of no organization's facilities, while others make use of material and technical conditions furnished by one or both of the parties pursuant to the contract. As regards the

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specific character of the results, some are later developments based on the employer's intermediate results, while some involve improvements of the original employer's technological results, some are technological programs that involve a synthesis of several scientific and technical results, while still others are entirely new inventions or creations made with the technical knowledge possessed by the inventor or creator. In short, when determining the rights to technological results a specific analysis must be made with reference to the different kinds of attendant conditions.

Article 10 of the Detailed Principles for Implementation of the Patent Law states that "inventions or discoveries made within a year after leaving a job, going on leave or being reassigned, which are related to the main duties performed for the original employer or to allocated tasks" are job-related inventions or creations. But it does not specify to which organization the relevant utilization or application rights belong when an employee moves from one organization to another, because this must be decided with reference to the specific circumstances.

Problems of rights of this kind involving technological results proceeding from technological contracts must be dealt with appropriately so that they are beneficial to personnel mobility, development of lateral ties and technological cooperation, dissemination and utilization of scientific and technological results, and the holding of spare-time jobs. Specifically, the technological results produced by an employee within one year of leaving one employer as a result of going on leave, retirement, discharge, resignation, transfer or retention without salary, and which are closely related to the duties performed for the original employer or are in the direct range of duties performed for the original employer, constitute job-related technological results. In cases where the individual did not produce the results in question by research undertaken at his own initiative for the new employer, the technological results have sprung from the duties performed for the original employer; but the original employer must give the individual compensation for the labor and all expenditures involved in producing the technological results. The rights of ownership of technological results which the individual produces for the new employer must be decided in terms of the working environment and facilities and the actual contributions of the original employer, the new employer and the individual--if, based on the specific circumstances, it can be affirmed that they are research results springing from the duties performed for the original employer or duties performed for the new employer, or that they spring from duties performed for both employers, specifying a method for distributing profits or payment of compensation.

Hunan Establishes New-Technology Development Zones

40080030b Tianjin JISHU SHICHANG BAO [TECHNOLOGY MARKET NEWS] in Chinese
24 Aug 88 p 1

[Article: "Hunan Province Decides To Establish a Series of Experimental
New-Technology Industry Development Zones"]

[Text] The Hunan Province government recently decided to establish several
experimental new-technology industry development zones at the province
level to serve as incubators for new technology enterprises in order to
develop province-level high-technology and new-technology industry devel-
opment networks.

Initially a rather high-level, integrated, demonstration Changsha Science
and Technology Development Zone will be established in Changsha to serve
as a high-technology and new-technology research center for the entire
province; subsequently, experimental zones based on large- and medium-
size enterprises, scientific research units, and specialized academies
and schools will be established in Zhuzhou, Xiangtan, Hengyang, Yueyang,
Shaoyang and Changde. Third, a group of small regional experimental
zones will be set up in Yiyang, Xinhua, Liliang, Yuanjiang and Linzhou
to modernize traditional economic mainstay industries with high technology
and new technology.

The main focus of the experimental zones will be the development of
information technology, new materials, biological engineering, new energy
souces and new conservation technologies, nuclear technology, laser
technology, and space technology; guided by the market and adhering to the
principle of providing their own operating expenses, choosing their own
organization, autonomous operation, and bearing their own profit and
loss, they will adopt a flexible operations system.

The Hunan provincial government has decided to adopt a series of prefer-
ential policies to encourage and support the establishment of experimental
zones and the development of new-technology industries. These measures
are as follows. (1) A reduced 15-percent income tax rate for new-
technology enterprises, and a reduced 10-percent income tax rate for
cases in which the direct exports exceed total enterprise output value
by 40 percent. Starting from the day on which the new technology

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enterprise is registered, the payment of income tax will be exempted for the first 5 years, while in the next 5 years half of the income tax will be exempted. (2) New-technology enterprises can rent buildings and procure land at a preferential price, and their capital construction projects will not be subject to a fixed capital investment figure, approval procedures will be simplified, and their construction will be given priority. (3) Instruments and equipment needed for new technology development that cannot be produced domestically and must be imported, as well as samples used for assimilation, will not be subject to import tax, and exported products will not be subject to the export tax. (4) Foreign exchange created by exports of the new technology enterprises will be retained in full by the enterprises for the first 3 years, and starting in the fourth year it will be divided between the locality and the enterprise in the ratio of 20 percent and 80 percent. This retained percentage of foreign exchange can be used as the enterprise desires. New-technology corporations whose total annual export exceeds US$300,000 will, with the approval of the provincial government, have the right to conduct foreign trade operations. (5) Commercial, scientific, and technical personnel of the new technology enterprises who go abroad more than once in the course of a year will receive their approval from the provincial government on the first occasion, and from the enterprise thereafter. (6) The depreciation period for instruments and equipment used for new-technology and related product development will be shortened to 4-7 years. (7) The new-technology enterprises will be allowed to established their own experimental prices for new-technology products that they have developed. (8) The new-technology enterprises can recruit scientific and technical personnel for specialized technical positions and can set salaries and bonuses for their personnel.

Baotou's Economic Development Under 'Spark Plan'

40080030c Tianjin JISHU SHICHANG BAO [TECHNOLOGY MARKET NEWS] in Chinese
31 Aug 88 p 1

[Article: "Baotou Makes Use of Its Scientific and Technical Advantages
to Vitalize Its Local Economy"]

[Text] In recent years, in close connection with its urgent key economic
problems, Baotou City has vigorously developed its technology market and
has comprehensively applied the "Spark Plan," promoting local economic
development.

Baotou City currently has 60,900 specialized technical personnel, and it
is a zone of economic and technological concentration in Nei Monggol [Inner
Mongolia]. How to make use of this advantage and cause the scientific
and technical contingent to enter the main battlefield of economic construc-
tion is a problem that Baotou City's science and technology committee has
been continuously investigating for several years. In 1985 it opened the
autonomous region's first parmanent technology market and set up scientific
research and production links. There are now more than 40 scientific and
technical development service organizations, using technology transfer,
consulting services, and similar forms to make technology permeate medium
and small enterprises in the banners, counties, and districts, thus pro-
moting local economic development. In the last 2 years the committee has
organized 8 groups of middle and high level scientific and technical
personnel, with a total of more than 100 members, to visit nearby banners,
counties, districts, and leagues to provide technical services and
technical consulation; these groups have taken on 61 difficult problems,
resulting in 72 contracts, 59 consulting services, and 12 technology
transfers. Last year a western Nei Monggol science and technology exchange
conference was held, during which 207 technology transfer and technological
cooperation arrangements were made, involving 3.852 million yuan of business,
and the movement of 64 persons. These activities helped promote commodity
conversion of Baotou City's scientific and technical results.

In addition, the committee made use of local resource advantages by
vigorously organizing Spark Plan implementation projects. The city's
scientific and technological committee raised 17.07 million yuan and
organized 10 Spark Plans, 7 of which are listed as national-level projects
and 3 as autonomous region-level projects. When all of these projects

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go into production, they will increase output value by 37.96 million yuan and generate more than 9 million yuan in taxes. Intermediate results have already been obtained on many of the projects.

In 1985, Engineer Wang Meihua, formerly of the Baotou Rare Earths Research Institute of the Ministry of Metallurgical Industry, opened her own research institute. The oilfield deep-well rare-earth strong ceramic anti-wax device series that she developed has attained an annual output value of 2.6 million yuan, has generated 600,000 yuan in profits, and has been listed as a national-level Spark Plan project. After these products were used on more than 400 oil wells at the Daqinq oil field, the annual economic effect per well reached 20,000 yuan. Last year the institute developed 1400 anti-wax devices, which are in extensive use at 14 oil fields. The Baotou No 2 Furs Plant has taken advantage of local resources of rare earths and furs, using rare-earth whiteners and new dye adjuvant technologies to produce goatskin drawing skins, with the result that the products rapidly made their way into international markets, producing 1.1 million yuan in profits taxes annually and creating US$700,000 in foreign exchange. This year, the two projects were recommended for national-level Spark Plan prizes.

Multicolored sheet steel paints are a national-level Spark Plan project taken on jointly by the Baotou Paint Plant and the Nei Monggol Petrochemical Research Institute. After importing the technology from the Pigments Institute of the Ministry of Chemical Engineering, the plant established China's first colored sheet steel paint production line, with an annual output of 800 tons.

Bazhong's Economic Development Under 'Spark Plan' Described

40080030d Tianjin JISHU SHICHANG BAO [TECHNOLOGY MARKET NEWS] in Chinese
31 Aug 88 p 1

[Article: "Bazhong County's Spark Plan Again Reaches a New Plateau"]

[Text] Bazhong County, Sichuan, which was listed as a national mountain
zone experimental integrated technology development center, last year
again reached a new plateau in Spark Plan development. The province's
22 Spark projects resulted in an industrial output of 18.647 million
yuan, 93 percent of the entire annual plan. The increase in profits tax
payments was 1,359 million yuan, and US$1.42 million in foreign exchange
was created; peasant income was 10.735 million yuan. Some 15 projects
countywide have met their technical and economic targets and have had
their products accepted; 70 percent of these are provincial level and local
projects.

In implementing the Spark Plan projects, the county focused on such main-
stay products as chopped meat, high-quality duck, and potted meat, with
an output value exceeding 10 million yuan. The province science and
technology committee organized the relevant departments to help the county
down plant effectively disseminate good quality duck breeding and devel-
opment of a series of down products, with the result that the Shu'e brand
down garments produced by the plant have won a good reputation on markets
in Beijing and Shanghai. These three mainstay projects alone produced
an output value of 17.735 million yuan, 92 percent of the province's
total Spark Plan project output value.

The units carrying out the Spark Plan projects all implemented a diverse
quota management responsibility system centered on benefit contracting
and in the last six months vigorously carried out technical training of
more than 1,700 people in village groups, in groups of rusticated young
intellectuals, and in demonstration scientific and technological households.

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Chinese Launch Vehicles Move Toward International Market

40080044 Beijing SHIJIE DAODAN YU HANGTIAN [MISSILES AND SPACECRAFT]
in Chinese No 5-6, May-June 88 pp 4-10

[Article by Zhang Zhiqing [1728 4949 1987] and Chen Shouchun [7115 1108 2797]]

[Text] Since China's aerospace industry was initiated in 1956, we have devoted more than 30 years of hard work to develop it. Today, we have established an independent and complete system of research and production, and also have made contributions which have attracted world-wide attention.

In June 1964, China launched its first launch vehicle designed and built domestically; in April 1970, China's first artificial earth satellite was launched by its own launch vehicle.

By March 1988, China had successively launched 22 satellites of various types, including 10 retrievable remote-sensing satellites used for scientific exploration and technology experiment, three technology experiment satellites launched by a single launch vehicle in September 1981, a geosynchronous communications satellite launched in April 1984, and two additional geosynchronous communications satellites launched respectively in February 1986 and March 1988.

These satellites were all launched into orbit by the family of Chinese-built Changzheng (CZ) launch vehicles (including the CZ-1, the CZ-2, the Fengbao (FB)-1, and the CZ-3) (see attached table of Chinese-built launch vehicles and artifical satellites). This demonstrates that China's satellite technology, launch vehicle technology, and sensor and control technologies have reached a level shared by other advanced nations of the world.

In October 1985, China's Minister of Aerospace Industry Li Xue announced that the CZ-2 and CZ-3 launch vehicles were entering the international market to seek satellite-launching business; China is also offering favorable conditions to user countries in an attempt to contribute to the international aerospace development.

During the past 2 years, over 20 national and international corporations or government organizations have initiated discussions with us about launching their satellites using Chinese launch vehicles or cooperating with us to launch satellites for a third party. Because of the established reliability of China's launch vehicles, and favorable pricing and insurance policies, flexible payment policy, and attractive safety and security policies, many foreign users have expressed strong interest in the family of Changzheng launch vehicles.

The purpose of this article is to give a brief introduction to the CZ-2 and CZ-3 launch vehicles which are already in commercial service, the early CZ-1 and FB-1 launch vehicles, and the high-powered CZ-4, CZ-3A, and CZ-2E launch vehicles which are currently under development.

The Changzheng-1

The Changzheng-1 (CZ-1) launch vehicle is also referred to as the LM-1 (Long March-1). It is China's first successful launch vehicle which began initial development in 1965 and became operational in 1970. On 24 April 1970, it successfully launched China's first artifical satellite, the Dongfanghong-1. On 3 March 1971, it successfully launched China's first scientific experiment satellite, the Shijian-1. However, since then, production and use of CZ-1 has been halted due to the lack of demand for launching small satellites.

The CZ-1 is a three-stage rocket; the first two stages used liquid-propellant engines (with UDMH as fuel and nitric acid as oxydizer), and the third stage uses a solid-propellant engine. The first and second stages of the rocket and the control system were developed based on the designs of China's medium-long range rockets, but the third stage was a newly developed solid-propellant rocket engine.

The rocket is 29.45 m long and has a maximum diameter of 2.25 m; its lift-off weight is 81.6 tons and the thrust at lift-off is 1020 k-N. The satellite cowling has a maximum diameter of 2.05 m, an effective static diameter of 1.56 m, and is 3.88 m high. It has the capability of launching a 300-kg payload into a 440-km circular orbit with an inclination of 70°.

The design and production unit of the CZ-1 rocket was the Beijing Wanyuan Industrial Corporation. In an attempt to improve CZ-1's performance, efforts were made by the Wanyuan Corp. to implementall-transistorized electrical systems and to miniaturize all system components. It also developed various kinds of new electronic components, semi-conductor devices and electronic measuring equipment, and applied a variety of new processing techniques. This provided a good foundation for subsequent development of other models of the family of Changzhen rockets.

Chinese-Built Launch Vehicles and Artificial Satellites

Sequence Number	Satellite Name	Launch Vehicle	Launch Site	Launch Date	Result
1.	Dongfanghong-1	Changzheng-1	Jiuquan Launch Center	1970.4.24	successful
2.	Space physics exploration satellite, Shijian-1	Changzheng-1	Jiuquan Launch Center	1971.3.3	successful
3.	Technology experiment satellite-1	Fengbao-1	Jiuquan Launch Center	1975.7.26	successful
4.	Retrievable remote-sensing satellite-1	Changzheng-2	Jiuquan Launch Center	1975.11.26	successful
5.	Technology experiment satellite-2	Fengbao-1	Jiuquan Launch Center	1975.12.26	successful
6.	Technology experiment satellite-3	Fengbao-1	Jiuquan Launch Center	1976.8.30	successful
7.	Retrievable remote-sensing satellite-2	Changzheng-2	Jiuquan Launch Center	1976.12.7	successful
8.	Retrievable remote-sensing satellite-3	Changzheng-2	Jiuquan Launch Center	1978.1.26	successful
9.	Space physics exploration satellite, Shijian-2	Fengbao-1	Jiuquan Launch Center	1981.9.20	successful
10.	Space physics exploration satellite, Shijian-2A	Fengbao-1	Jiuquan Launch Center	1981.9.20	successful
11.	Space physics exploration satellite, Shijian-2B	Fengbao-1	Jiuquan Launch Center	1981.9.20	successful
12.	Retrievable remote-sensing satellite-4	Changzheng-2	Jiuquan Launch Center	1982.9.9	successful

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No.	Satellite	Launch Vehicle	Launch Center	Date	Result
13.	Retrievable remote-sensing satellite-5	Changzheng-2	Jiuquan Launch Center	1983.8.19	successful
14.	Communications and technology experiment satellite	Changzheng-3	Jiuquan Launch Center	1984.1.29	partially successful
15.	Experimental communications satellite	Changzheng-3	Jiuquan Launch Center	1984.4.8	successful
16.	Retrievable remote-sensing satellite-6	Changzheng-2	Jiuquan Launch Center	1984.9.12	successful
17.	Retrievable remote-sensing satellite-7	Changzheng-2	Jiuquan Launch Center	1985.10.21	successful
18.	Operational communications satellite	Changzheng-3	Jiuquan Launch Center	1986.2.1	successful
19.	Retrievable remote-sensing satellite-8	Changzheng-2	Jiuquan Launch Center	1986.10.6	successful
20.	Retrievable remote-sensing satellite-9	Changzheng-2	Jiuquan Launch Center	1987.8.5	successful
21.	Retrievable remote-sensing satellite-10	Changzheng-2	Jiuquan Launch Center	1987.9.9	successful
22.	Operational communications satellite	Changzheng-3	Jiuquan Launch Center	1988.3.7	successful

The Changzheng-2

The Changzheng-2 (CZ-2) launch vehicle is also referred to as the LM-2 (Long March-2). It is another launch vehicle designed to launch China's low-earth-orbit satellites. A technology feasibility study of the rocket began in 1964, full-scale development began in 1970, and it became operational in 1974. On 15 November 1974, it was used to launch China's first remote-sensing satellite, but due to a broken lead wire in the control system, the flight test failed. As a result of this failure, a series of measures were taken to improve the reliability of the rocket, and on 26 November 1975, it successfully launched China's retrievable remote-sensing satellite used for scientific exploration and technology experiment. By September 1987, the CZ-2 had successively launched 10 retrievable satellites. The success of these launches proved that the CZ-2 is a well-designed rocket, and its performance and reliability are quite satisfactory.

The CZ-2 is a two-stage liquid-propellant rocket; the engine fuel is UDMH, and the oxydizer is nitrogen-tetroxide. The first stage has four gimballed engines with a total thrust of 2,700 k-N; the second stage has main engine with a thrust of 720 k-N and a floating engine.

The rocket is 32.57 m long and has a maximum diameter of 3.35 m; its lift-off weight is 191 tons and the thrust at lift-off is 2,700 k-N. It has the capability of launching a 1.8-2.0-ton payload into a 175-400 km orbit with an inclination angle between 70° and 57°.

The CZ-2 has two different types of cowlings: one has a maximum diameter of 2.2 m, an effective static diameter of 1.8 m, and a height of 2.5 m; the other has maximum diameter of 3.35 m, an effective static diameter of 3 m, and a height of 5 m. These dimensions can be redesigned according to user requirements.

In developing the CZ-2, a series of advanced technologies have been used. For example, a platform computer design is used in the guidance system, and floating engines are used to provide the control force; regenerative pressurization technique is used in the propellant supply system; and high-strength aluminum-copper alloy is used to build a light-weight propellant tank. In particular, a new high-thrust rocket engine has been developed which greatly increases the launch capability of the rocket.

The CZ-2 has enough launch capability to carry other payloads in addition to the scientific exploration and technology experiment satellite. We can design the cargo bay according to the payload requirement and provide an orbit transfer module which is capable of injecting the satellite or payload into its final orbit through one or two orbit transfer maneuvers.

Because of the CZ-2's reputation as a highly reliable launch vehicle, many research institutions and corporations around the world have shown strong interest in using the CZ-2 to launch their scientific experiments

into space. In August 1987, the space division of the French Matra Industrial Group successfully launched two micro-gravity experiments on the CZ-2 (one was an experiment of growing seaweed in space, the other was an experiment of micro-gravity measurements). Subsequently, the European Space Agency, three French space research centers, and other French, German, and Belgian companies had expressed interest in cooperating with us to put micro-gravity experiments into space using the CZ-2.

The Fengbao-1

In the early 70's, while the CZ-2 launch vehicle was being developed in Beijing, another launch vehicle was being developed in Shanghai; this launch vehicle was based on the CZ-2 design and was given the name Fengbao-1 (or FB-1).

In developing the FB-1, some modifications were made to the CZ-2 design due to constrains in Shanghai's production capabilities; in particular, significant modifications were made to the second-stage floating engine. Unfortunately, these modifications degraded the original design margin; furthermore, ground testing was inadequate, and strict quality control and system management was lacking; as a result, the first satellite launched by the FB-1 was a failure. An investigation of the failure showed that it was caused by a sudden drop in the thrust of the second-stage floating engine. In an effort to improve the reliability of the FB-1, it was decided to replace this engine with the second-stage engine of the CZ-2, thus bringing FB-1's design configuration even closer to that of CZ-2.

In 1981, after a successful launch of three satellites by the FB-1, a decision was made to stop production of the FB-1 in Shanghai in order to devote Shanghai's resources to the development of the first and second stages of the Changzheng-3 launch vehicle. These two stages are based on the design of the CZ-2, so they would be compatible with the third stage of the CZ-3 being developed in Beijing.

The FB-1 had six satellite-launch tests four of which were successful; during these tests, three space physics exploration satellites and three technology experiment satellites were injected into their pre-designated orbits. The causes for the two failed tests have been identified and corrective measures have been taken.

The four successful satellite launches and three other successful flight tests have sufficiently demonstrated its design performance and reliability. Since 1980 when the CZ-2 second-stage engine was used on this rocket, it had four consecutive successful satellite launches.

Figure 1. The Changzheng Family of Launch Vehicles

|	CZ-1	CZ-2	CZ-3	CZ-4	CZ-3A	CZ-2E
length (m)	28	35	44.6	42	52	48~50
diameter (m)	2.25	3.35	3.35	3.35	3.35	3.35

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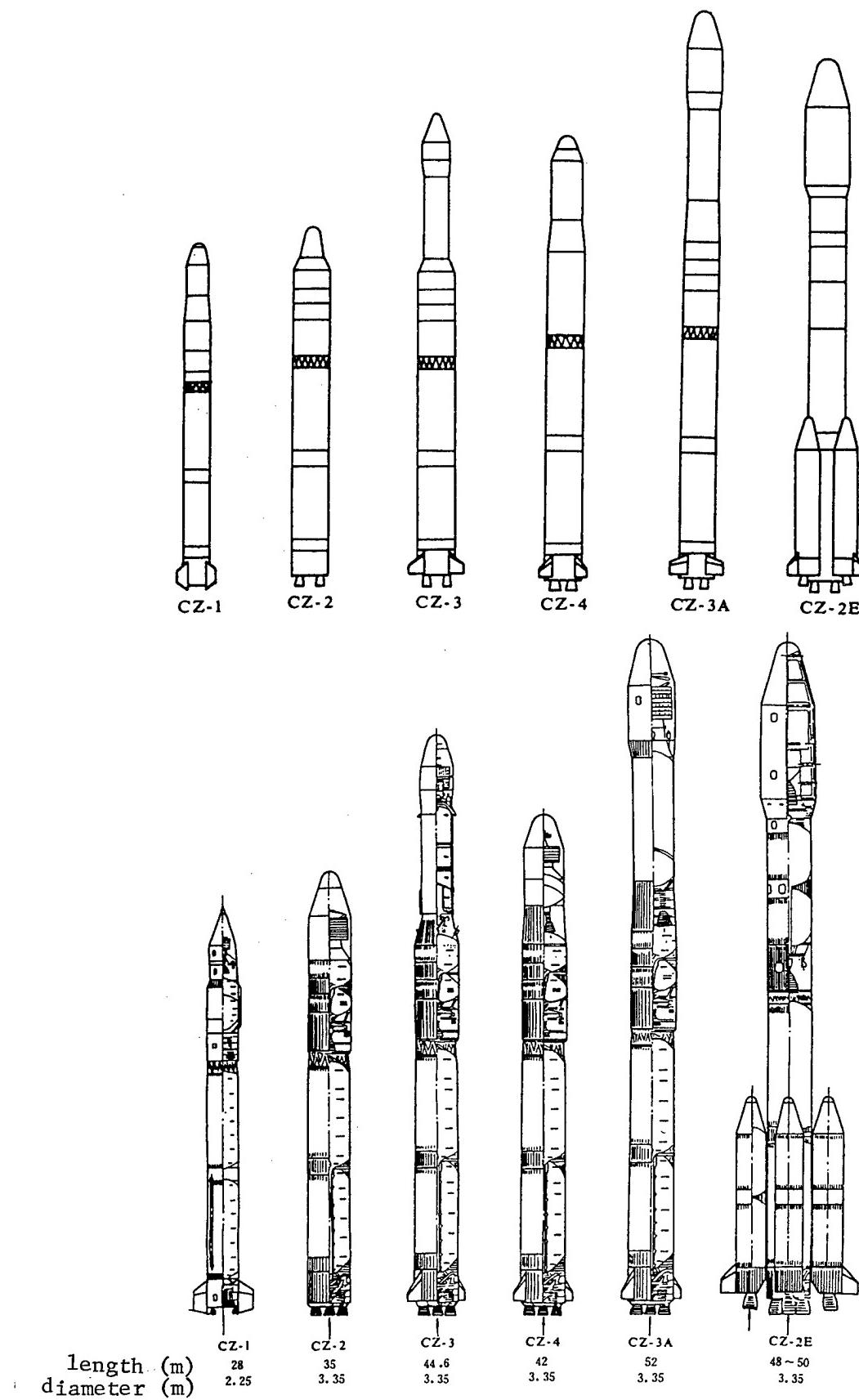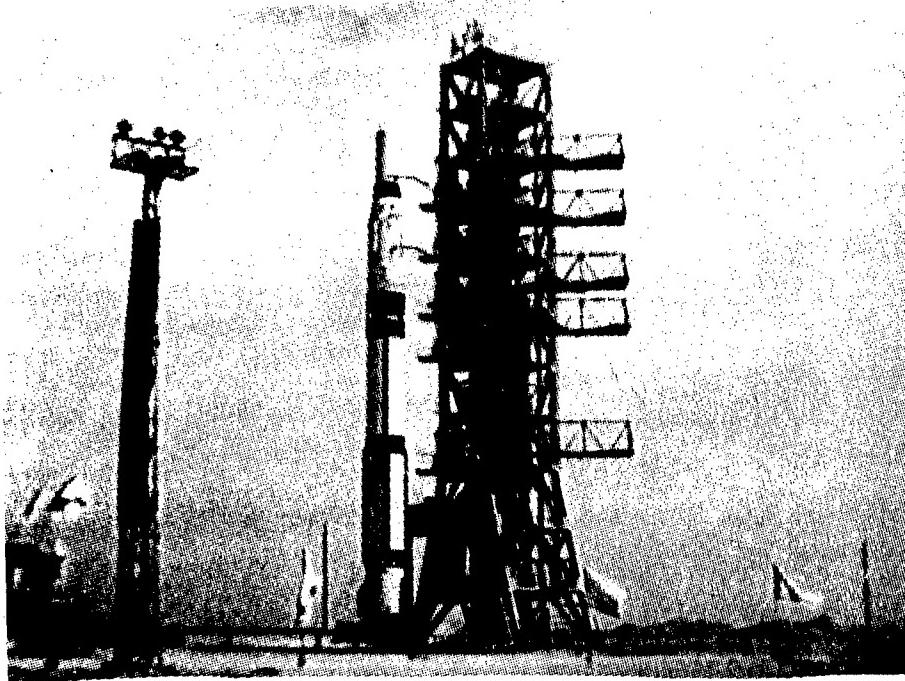
The Changzheng-1 (CZ-1) Launch Vehicle
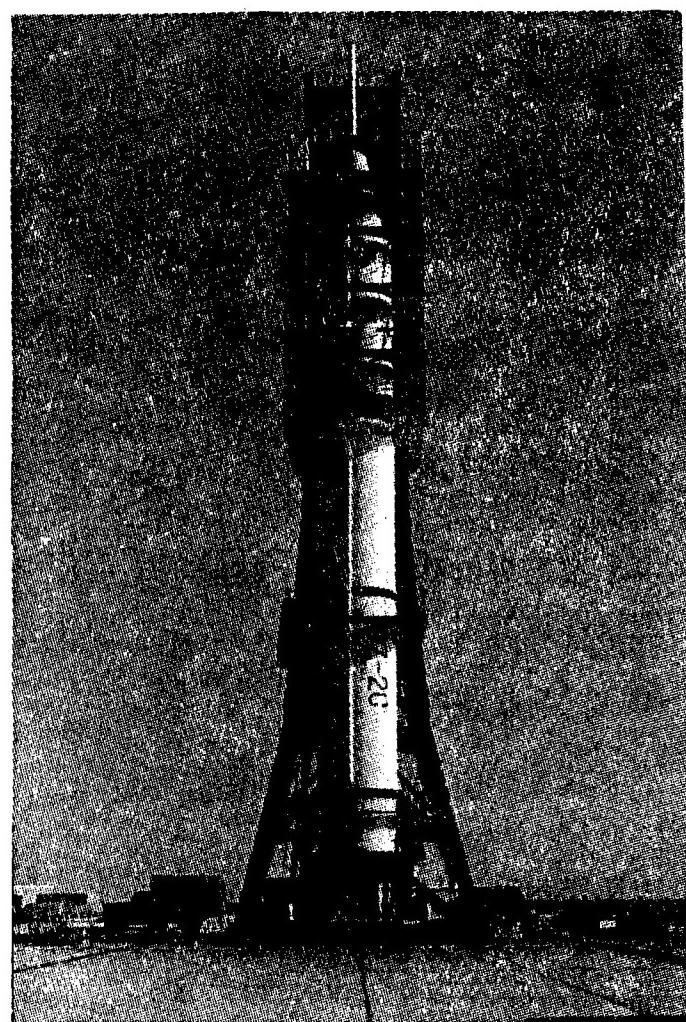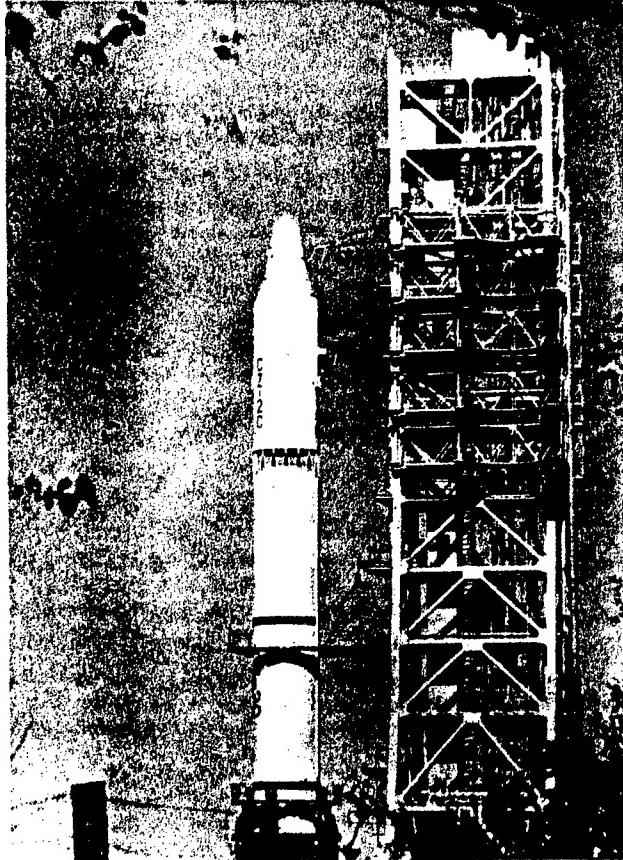
The Changzheng-2 (CZ-2) Launch Vehicle
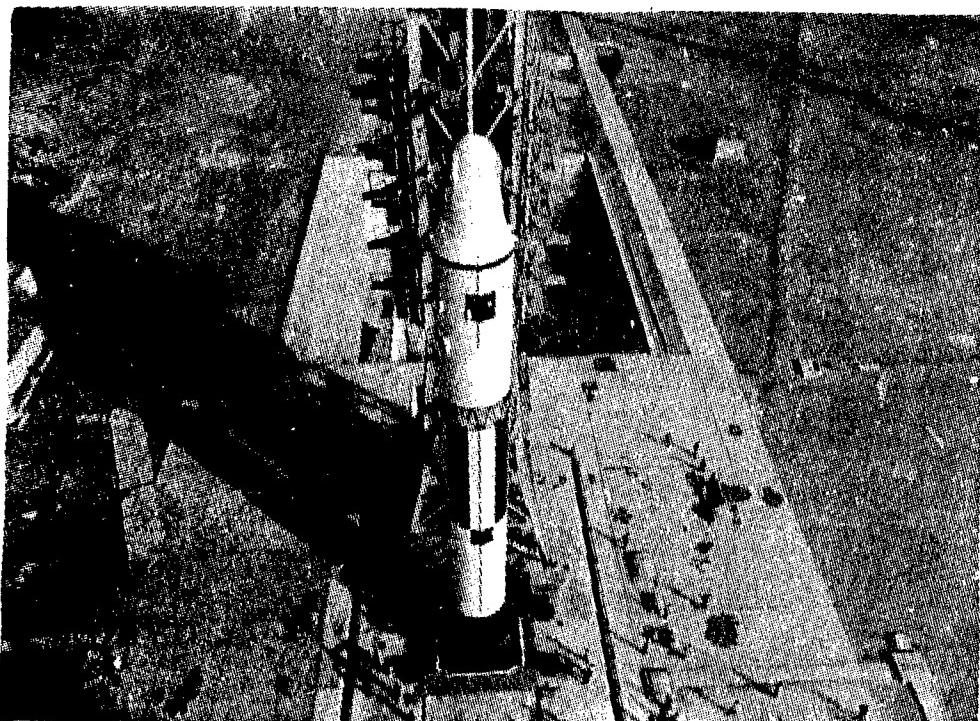
The Changzheng-3

The Changzheng-3 (CZ-3) launch vehicle is also referred to as the LM-3 (Long March-3). It was developed based on the CZ-2 design to meet the requirements of launching geosynchronous communications satellites for China's telecommunications and broadcast industry.

The first and second stages of the CZ-3 were developed by modifying the first two stages of the CZ-2; the third stage was a newly designed low-temperature, high-energy liquid-hydrogen and liquid-oxygen engine. In addition to the use of low-temperature propellant, the third-stage engine has also incorporated the feature of secondary ignition, which allows accurate injection of communications satellites into geosynchronous transfer orbits in the Pacific Ocean.

The CZ-3 launch vehicle 44.25 m long; the first two stages have a diameter of 3.35 m, and the third stage has a maximum diameter of 2.25 m. The lift-off weight is 202 tons, and the thrust at lift-off is 2900 k-N (its launch capability is shown in the following table).

Launch Capability of the CZ-3

Orbit	Height (km)	Inclination (deg)	Launch capability (kg)
Sun-synchronous	200	96.34	4100
Sun-synchronous	400	97.05	3800
Sun-synchronous	600	97.81	3500
Sun-synchronous	800	98.63	3100
Sun-synchronous	1000	99.51	2700
Geosynchronous (tranfer orbit)Hp/Ha	200/35786	31.5	1400
Geosynchronous (transfer orbit)Hp/Ha	400/35786	31.5	1800

The rocket has two different types of satellite cowlings: one has a maximum diameter of 2.6 m, an effective static diameter of 2.32 m and a height of 4.06 m; the other has a maximum diameter of 3 m, an effective static diameter of 2.72 m and a height of 5.33 m. In addition, a special cowling can be designed according to user specifications.

The CZ-3 launch vehicle began development in 1977 and became operational in 1984.

On 29 January 1984, the CZ-3 launched its first satellite. During the flight, the first and second stages operated normally; the third-stage ignition and the coast phase were also executed normally to achieve first cosmic velocity. But after secondary ignition, the engine thrust only reached 90 percent of its design value, and subsequently the pressure in the combustion chamber suddenly dropped. After the malfunction, the computer on board the third-stage rocket was able to maintain the flight attitude, complete the separation of the satellite from the rocket, and inject the satellite into a low-earth orbit (with an apogee of 6,480 km and a perigee of 400 km). The land-based tracking stations and the ship-borne tracking radars also succeeded in collecting all the measured data and completing the intended communication and telecast experiments. But since the satellite was not injected into an orbit with an apogee of 35,786 km, the test objectives were not completely satisfied, and the test was only partially successful. A short time later, this problem was corrected by making a modification to the hydrogen-oxygen engine.

On 8 April 1984, 70 days after the first launch, the CZ-3 attempted a second launch; this time it succeeded in injecting an experimental communication satellite into the designated geosynchronous transfer orbit, and on 16 April the satellite was accurately positioned at 125° East Longitude above the Equator.

On 1 February 1986, the CZ-3 successfully launched its third payload and injected an operational communication satellite into a designated geosynchronous transfer orbit. On 19 February, the satellite was injected into a geosynchronous orbit at 103° East Longitude above the Equator.

On 7 March 1988, the CZ-3 successfully launched its fourth payload--an operation communication satellite, and it was injected into geosynchronous orbit on 22 March and positioned at 87.5° East Longitude above the Equator.

These results demonstrated that we have the capability to analyze the causes of the malfunctions and take effective corrective measures to improve the reliability of the rocket.

Evaluation of the technical data shows that the theoretical orbit injection accuracies of the CZ-3 are as follows:

Δa = $\pm$ 50 km (deviation in semi-major axis)
Δi = $\pm$ 0.07 (deviation in orbit inclination)
Δh_p = $\pm$ 6 km (deviation in perigee)
$\Delta \omega$ = $\pm$ 0.29 deg (deviation in argument of perigee)
$\Delta \Omega$ = $\pm$ 0.14 deg (deviation in right ascension of ascending node)

In fact, measured results from the three successful CZ-3 launches showed that actual deviations in the orbit parameters were all smaller than the above values, and the measured data were highly repeatable.

Currently, the CZ-3 has become China's primary launch vehicle on the international satellite-launch market, and efforts are made to expand its service to foreign users.

Long March-4
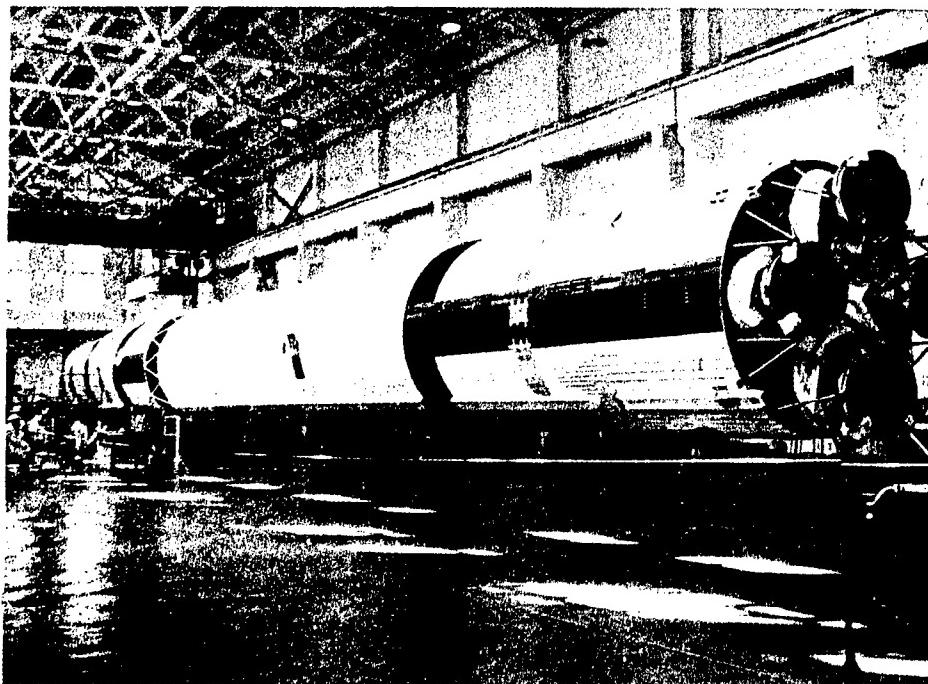
The Changzheng-4

The Changzheng-4 (CZ4) launch vehicle is also referred to as the LM-4 (Long March-4). It is another large launch vehicle designed to launch satellites into sun-synchronous orbits or geosynchronous transfer orbits. The responsibility for its development was assigned to the Shanghai Bureau of Aeronautics and Astronautics.

The CZ-4 is a three-stage liquid-propellant rocket; all three stages use the conventional propellant--UDMH and nitrogen tetroxide.

The three stages of CZ-4 are based on the CZ-2 design; the third stage consists of two 50 k-N engines. The rocket is 42 m long; the first two stages have a diameter of 3.35 m and the third stage has a diameter of 2.9 m. The lift-off weight is 249 tons and the thrust at lift-off is 2900 k-N. There are two types of cowlings: one has a maximum diameter of 2.9 m, an effective static diameter of 2.36 m, and a height of 2.91 m; the other has maximum diameter of 3.35 m, an effective static diameter of 3 m, and a height of 6.5 m. The dimensions of the cowling can also be designed according to user specifications.

The launch capability of the CZ-4 for a sun-synchronous orbit is as follows: at an altitude of 600 km, the effective payload is 2.5 tons; at an altitude of 900 km, the effective payload is 1.4 tons.

The Changzheng-3A

The Changzheng-3A (CZ-3A) launch vehicle is also referred to as the LM-3A. It is a powerful rocket designed to launch China's new high-capacity communications and broadcasting satellite, the Dongfanghong-3, and to compete for business on the international market to launch large payloads. At an inclination of 31.1°, it has the capability of launching a 2.5-ton payload into a geosynchronous transfer orbit (GTO).

The Changzheng-3A is an improved version of the CZ-3; specifically, improvements have been made in the control system in the area of digitization and miniaturization; also, the three-axis floating platform is replaced by a four-axis flexible platform. The newly designed third-stage engine consists of two 78 k-N bi-directional gimballed engines to achieve higher efficiency. The diameter of the third sub-stage is increased from 2.25 m to 3 m.

The rocket is 52 m long; the first and second stages have a diameter of 3.35 m, and the third stage has a diameter of 3 m. The cowling has a maximum diameter of 3.35 m, an effective static diameter of 3 m, and a height of 5.25 m. The cowling dimensions can also be designed according to user specifications.

The Long March-4 Launch Vehicle
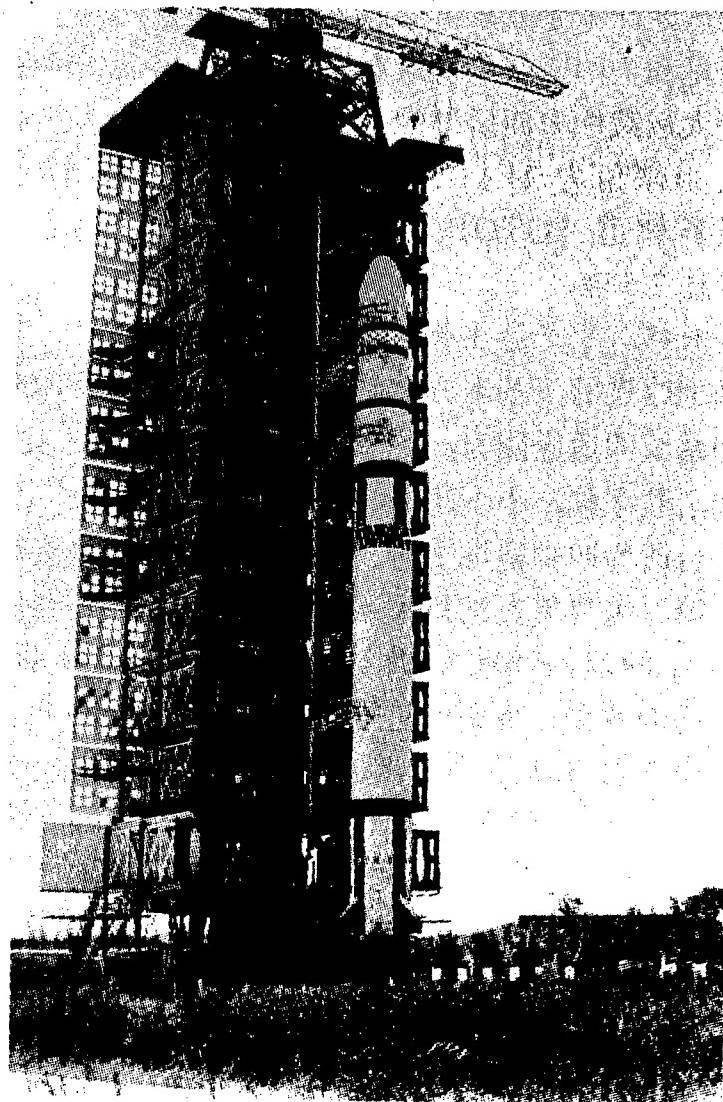
To increase its launch capability, the CZ-3A can be supplemented with boosters. There are three different booster designs whose GTO launch capabilities are respectively as follows:

1. With 4 solid boosters, the launch capability is 2.8 tons.
2. With 2 liquid boosters, the launch capability is 3.8 tons.
3. With 4 liquid boosters, the launch capability is 4.5 tons.

The Changzheng-2E

The Changzheng-2E (CZ-2E) launch vehicle is also referred to as the LM-2E. It is a two-stage rocket with 4 liquid-propellant boosters;it is primarily designed to meet the needs of China's growing space industry and the needs

of the international satellite-launch market. It can launch a 8.8-ton effective payload into a 200-km low-earth orbit with an inclination of 28.5°.

The CZ-2E has the unique feature that it is compatible with many upper stages built by other countries. For example, it can be used in conjunction with the PAM-D3, the PAM-D4, the AMS, the SCOTS and the STV; at the present time, its GTO launch capability is approximately 2.5 tons.

The CZ-2E is developed by lengthening the first and second stages of the highly successful CZ-2, and strapping four liquid-propellant boosters to it. The liquid boosters are made from the CZ-1 body which has a diameter of 2.25 m. The engines are identical to those of the core stage; the boosters are separated from the main rocket body once the propellant is exhausted.

The rocket is 51 m long; the first and second stages have a diameter of 3.35 m, and the booster has a diameter of 2.25 m. The lift-off weight is 430 tons, and the thrust at lift-off is approximately 5,900 k-N.

The cowling has maximum diameter of 4.2 m and an effective static diameter of 4 m.

Development of the CZ-2E is expected to be completed and the launch vehicle ready for service by 1990.

References

1. "The Aerospace Industry in Today's China," Chinese Social Sciences Publisher.

2. Zhang Zhiqing, Chen Shouchun, "China's Aerospace Industry and Its Potential To Provide International Service."

3. Xie Guangxuan, Zhang Zhiqing, "The Chinese Changzheng-3 Launch Vehicle" (Technical Proceedings of the Geneva International Space Technology Conference, May 1986).

4. "China's Aerospace Technology and Products," Great Wall Industrial Corporation.

Prospects for China's Launch Vehicles in Competitive Market

40080043 Beijing SHIJIE DAODAN YU HANGTIAN [MISSILES AND SPACECRAFT] in Chinese No 7, Jul 88 pp 8-11

[Excerpt]   IV.   The Challenge Facing China Today

Aerospace technology is recognized internationally as one of the modern high technologies. Its unique features are: large investment, high risk, with potentially high social and economic payoffs. The governments of the United States, France, Japan, and the Soviet Union have all adopted a series of protectionist policies to support the development and trade. This resulted in fierce competition among the nations and created a politically oriented aerospace market.

The challenge facing China today is that it must take the initiative to compete and try to expand its share on the international market. After more than 30 years of dedicated effort in developing this high technology, China is in a good position to compete with the United States, France, Japan, and the Soviet Union. Japan is already challenging other nations even though its H-2 rocket is still in the blueprint stage. Given the foundation China has built over the years and the current advantage it has in this area, we run the risk of losing an excellent opportunity if we do not actively compete now. China's ability to compete on the other hand, depends on our decision to accelerate the development of the new CZ-2E launch vehicle. Otherwise, we will lose our competitive edge and be forced out of the international market.

To be competitive on the international market also depends on other factors such as pricing policy, payment policy, insurance policy, investment policy, advertising and sales policy, customs import and export policy, tax policy, middle-man policy, technology development policy, and service policy. China has some unique advantages in all of these areas.

The French Ariane Aerospace Co. plans to launch 42 satellites using the Ariane-4 rocket. Among these 42 satellites, 12 are of the heavy weight class. But they account for only 32 percent of the market; we can still pursue the other 68 percent of the market share.

The U.S. Titan rocket has already been scheduled to carry 28 military cargoes before 1992; also, it is not very competitive because its commercial launch cost is more than twice the cost of other countries.

The Thor Delta-2 rocket has the capability of launching a 1,816-kg payload into a geosynchronous transfer orbit, and the U.S. Air Force has already signed up for 10 launches; but it is not a threat to the CZ-2E rocket.

The Soviet Proton rocket has difficulty launching satellites into 28°-inclination orbit because of its high-latitude launch site. If the satellite is launched into a 51.6° transfer orbit, then its life-span will be greatly reduced. Therefore, the Proton rocket generally launches a satellite directly into a geostationary orbit, which limits its ability to satisfy different user requirements. Furthermore, due to political reasons, the United States and Western Europe will try very hard to resist the Soviet Union's attempt to enter the aerospace market.

Japan's H-2 rocket is still under development; it does not have the technical reputation that this country has.

China's CZ-2E rocket has already attracted the attention and interest of many satellite manufacturers and users. Since 1986, we have been contacted by the following companies: Hughes, RCA, Ford, BAe, McDonnell-Douglas, Boeing, the Canadian Communication Satellite Corp., and the Australian Communication Satellite Corp. They have entered negotiations with us to launch a total of dozens of satellites. They believe that the CZ-2E rocket is well suited for launching the new-generation satellites with variable orbits. There have also been a number of business meetings and technical discussions.

Based on the above discussion, we can conclude that the international aerospace market does exist, and we are optimistic about our chances of entering this market in the future. Although the competition will be tough, and we will no doubt encounter some difficulties, but as long as we try hard to develop the market, it is very likely that we will be awarded our share of the contracts.

However, we must take immediate steps to solve the problem in front of us: First, we must take the necessary measures to accelerate the development of the new CZ-2E rocket; second, we must enter the competitive market armed with an open-door policy and our existing technical advantages. We cannot afford to apply the concept of national planned economy in a highly competitive international market; otherwise we may lose this valuable opportunity. This is an important decision facing China's aerospace industry; it deserves everyone's close attention.

CZ-2E

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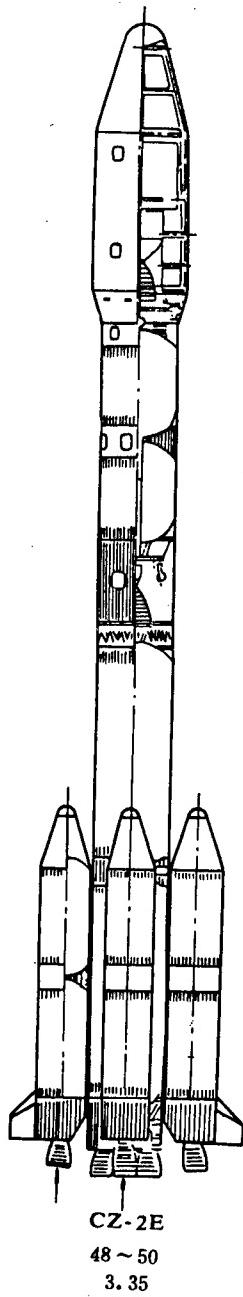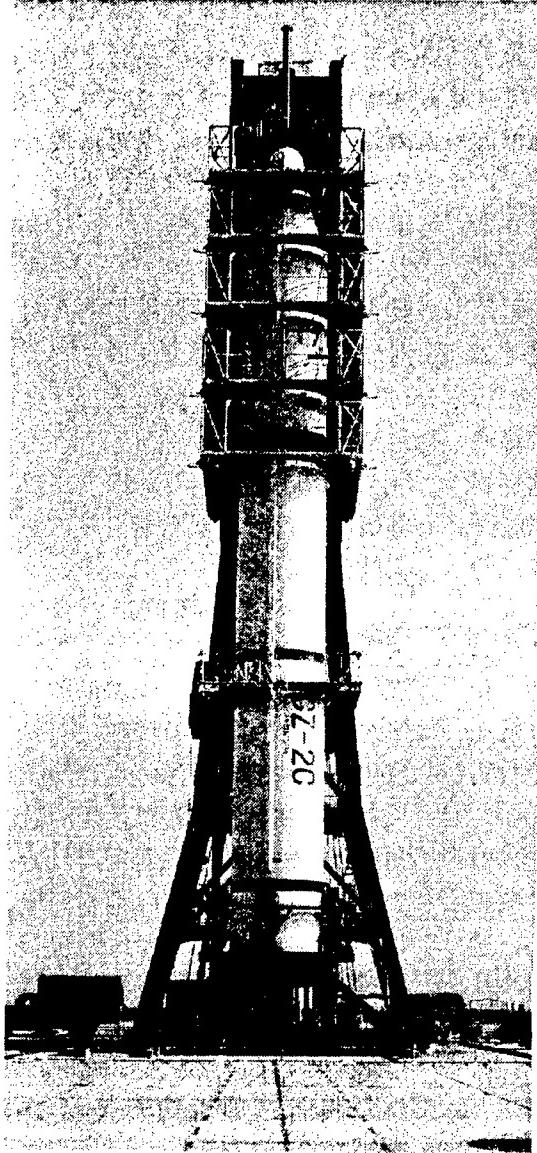
The Chinese-Developed CZ-2 and CZ-2E Launch Vehicles

Research on Carbon Fiber Surface Treated Continuously By Air-Cooled Plasma

[Article by Zhang Xiubin [1728 4423 2430], Zhang Zhiqian [1728 1807 6197], and Wei Yuezhen [7614 2588 6297] of the Harbin Institute of Technology, Polymer Materials Research and Teaching Section; manuscript received January 1988]

[Text] Abstract

We used glow discharge technologies for continuous air-cooled plasma surface treatment of a carbon fiber surface. The results showed that plasma treatment can greatly improve adhesion between the carbon fiber and matrix. The interlaminar shear strength (ILSS) of the composite material increased from 641 kg/cm^2 before treatment to 871 kg/cm^2, attaining the performance indices of Japan's T-300 [fiber]. This technique is simple, easy to use, economical, safe, poses no hazard to the public, and can be used in conjunction with carbon fiber production lines.

I. Introduction

Carbon fiber reinforced composites, with their high specific strength and specific modulus, resistance to corrosion, and other excellent properties, are now widely used in a variety of realms like astronautics, aviation, the chemical industry, medicine, sports equipment, and others[1-3]. They have developed extremely quickly in recent years. World carbon fiber production and demand have grown year after year, and its properties also have improved gradually[4]. However, the smooth and highly inert surface of carbon fiber results in poor adhesion with the matrix resin. The resulting low ILSS of the composites has restricted their range of applications. This has led many people to do research in this area from the 1960's to today and suggest various effective surface treatment methods. The openly reported ones include: surface oxidation[5], surface coating[6], cold plasma surface treatment[7], and others. Each of these treatments has shortcomings but cold plasma surface treatment produces the best overall results:

1. Cold plasma treatment is limited to modification of the fiber surface and has little effect on the properties of the fiber itself. This avoids the shortcoming of serious damage to fiber strength by the gas phase oxidation method.

2. Cold plasma surface treatment produces good results and usually takes only a few minutes. It can increase the composite ILSS by over 200 kg/cm^2.

3. Cold plasma treatment involves only a gas-solid reaction and no liquids. It is economical, safe, and poses no public threat, whereas the surface coating method usually is more expensive.

4. Cold plasma treatment is completed in one step, avoiding the complex washing, drying, and other measures involved in liquid phase oxidation.

However, cold plasma generation requires a vacuum environment. At present it is limited mainly to intermittent treatment and cannot be used in industrial production. This makes research on continuous cold plasma surface treatment for use in conjunction with carbon fiber production lines extremely important.

II. Material and Treatment Techniques

1. Raw material

1) Carbon fiber; High strength carbon fiber (3,000 strands/bundle) produced by the Jilin Carbon Plant, containing more than 90 percent carbon.

2) Matrix material: The matrix material is bisphenol A epoxy resin of this mixture:

Component	Content
E-51	33.30 g
BF$_3$ ethylamine	1.00 g
Acetone	27.00 ml

2. Cold plasma continuous treatment facility:

1) Cold plasma generator: Frequency 13.56 MHz, output power 0 to 1,000 W

2) Sealer: Our own design and manufacture, capable of simultaneously passing 1 to 50 fiber bundles (3,000 strands/bundle) in parallel, vacuum of 5 x 10^{-1} to 5 x 10^{-2} mm Hg.

The treatment facility and flow process are shown in Figure 1.
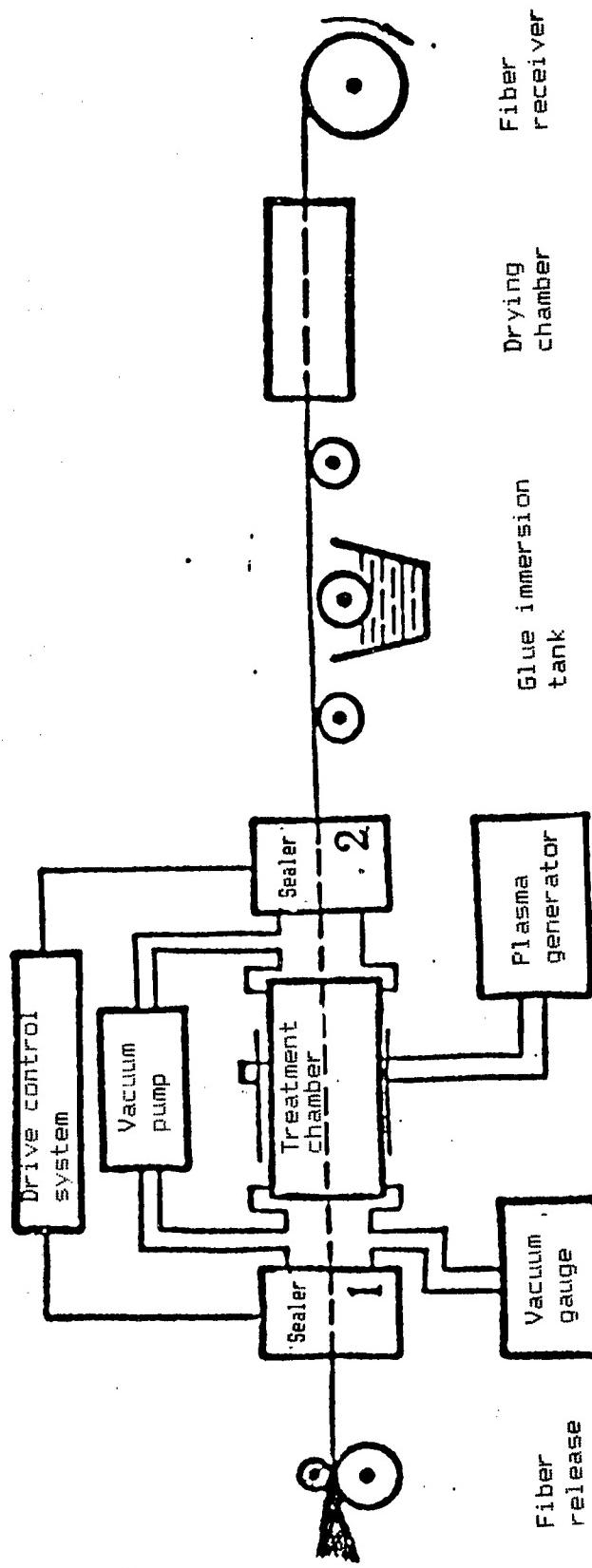
Figure 1. Continuous Treatment Facility and Flow Process

Fiber release

Fiber receiver

Drying chamber

Glue immersion tank

Sealer 2

Plasma generator

Drive control system

Vacuum pump

Treatment chamber

Sealer 1

Vacuum gauge

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3. Treatment technique:

Eight bundles of fiber were drawn through the sealer into the plasma treatment chamber at a set speed for air-cooled plasma surface treatment. The treated carbon fiber then passed through another sealer into a glue immersion tank for glue immersion. Then it passed through a constant temperature tube (temperature 100°C) to remove the acetone solvent in the liquid glue. Finally, it was wound on a fiber receiving spool. The entire process operated continuously. When the spool contained enough fiber it was cut off and placed into the mold. It was pressurized and solidified on a press and made into a unidirectional fiber reinforced composite.

4. Measurement of composite ILSS

It was tested using the three-point flexure method and had a span-height ratio of 5:1. The sample dimensions were 22 x 6.5 x 2 mm. The pressure loading head had a radius of 2 mm and the loading speed was 15 mm/minute.

III. Experimental Results and Discussion

1. The results of carbon fiber unidirectional reinforced composite ILSS tests are shown in Table 1.

Table 1. Effects of Plasma Input Power on Composite Material ILSS

Plasma input power (W)	$\overline{ILSS_n}$ (kg/cm²)	$\dfrac{\overline{\Delta ILSS_n}}{\overline{ILSS_n}}$ %	$\sigma_{n-1}/\overline{ILSS_n}$	Glue content (%)
Unprocessed	641	0	0.045	
1800	761	18.7	0.043	
1860	828	29.0	0.022	
1910	838	30.7	0.050	
1990	871	35.9	0.015	28±2
2050	818	27.6	0.027	
2100	835	30.3	0.028	
2170	798	24.5	0.041	
2240	788	22.9	0.091	
2300	731	14.0	0.094	

Note: Treatment time t = 2 min., pressure p = 0.4 mm Hg

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Table 1 shows that air-cooled cold plasma continuous carbon fiber treatment obviously increases the composite ILSS. The maximum value was 871 kg/cm^2, which is 230 kg/cm^2 higher than the untreated variety. The maximum value reached the level of Japan's T-300 carbon fiber and discrete coefficients decreased. The data in Table 1 were used to plot the ILSS-input power curve.

Figure 2 shows clearly that ILSS follows the laws of changes in plasma input power. At input power below 1,990 W, ILSS increases with increased input power. At input power above 1,990 W, ILSS decreased with increased input power.
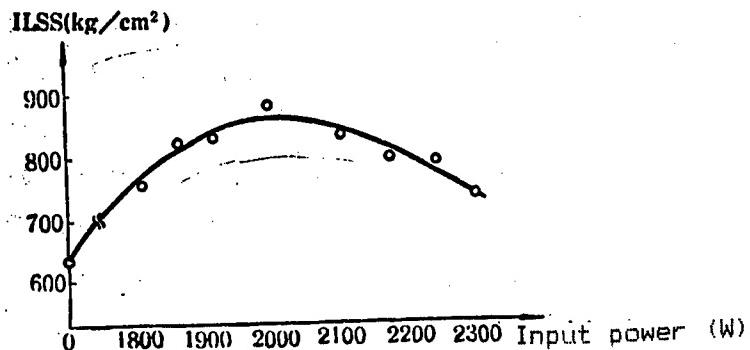

Figure 2. Effects of Plasma Input Power on Composite Material ILSS

2. Electron microscope analysis of fiber surface shape and the shape of composite fractures

A J x A-840 scanning electron microscope was used to examine fractures in treated and untreated carbon fiber and the unidirectional reinforced composite. The results are shown in Figures 3 and 4 [not reproduced].

There was an obvious deepening of fiber surface grooves after plasma treatment. This was caused by bombardment etching of the carbon fiber surface by active components in the plasma. On the one hand, it expanded the adhesion boundary between the fiber and matrix resin. On the other hand, it increased mechanical integration between the fiber and matrix (also called a "nail" effect). Both of these things increase the strength of adhesion between the fiber and matrix.

Plasma treatment definitely improved adhesion between the fiber and matrix resin. There was severe separation of the fiber and matrix in carbon fiber which did not undergo plasma treatment, and no resin adhered to the fiber surface. After plasma treatment, the fractures assumed fault shapes, very little fiber was pulled out of the resin, and obvious resin adhered to the fiber surface.

3. Fiber surface ESCA analysis

An ESCALAB-MK II X-ray photoelectron spectrometer was used for quantitative analysis of element composition and oxy radical content of the pre- and post-treatment fiber surface, with the results shown in Tables 2 and 3.

Table 2. Carbon Fiber Surface ESCA Elemental Analysis

Input power (W)	Carbon content (percent)	Oxygen content (percent)	Nitrogen content (percent)
Unprocessed	95.14	3.53	1.33
1860	94.29	3.81	1.90
1990	93.67	4.33	2.00
2240	93.98	4.62	1.40

$t = 2\,\text{min}$ $p = 0.4\,\text{mmHg}$

Table 3. Carbon Fiber Surface ESCA Oxy Radical Content Analysis

Input power (W)	C_{1S}							
	$>\!C\!-\!OH$		$>\!C=0$		$-C\!\!\stackrel{O}{\diagdown_{OH}}$		$>\!C\!-\!H$	
	$E_b(\text{ev})$	Content (percent)	$E_b(\text{ev})$	Content (percent)	$E_b(\text{ev})$	Content (percent)	$E_b(\text{ev})$	Content (percent)
Unprocessed	286.9	19.01	288.1	6.61	289.4	0.83	285.0	73.55
1860	286.9	18.18	288.1	7.41	289.4	1.60	285.0	72.79
1990	286.9	18.72	288.1	6.12	289.4	2.81	285.0	72.35
2240	286.9	22.18	288.1	5.30	289.4	0.94	285.0	71.58

$t = 2\,\text{min}$, $p = 0.4\,\text{mmHg}$

The results of elemental analysis showed an increase in both oxygen and nitrogen content after cold plasma treatment of the carbon fiber, indicating oxidation of the carbon fiber surface. The results of analysis of the C_{1s} absorption peak to determine oxidation products and content showed that the oxygen exists mainly as $>C-OH$, $>C=O$, $-C{\overset{O}{\underset{OH}{\diagup\diagdown}}}$ and other oxy radicals.

Moreover, all these values increased to varying degrees after plasma treatment. The entry of these oxy radicals into the carbon fiber surface aided in improving adhesion between the fiber and matrix. On the one hand, physical and chemical interaction may occur between these oxy radicals and amino groups in the matrix. On the other hand, oxy radical entry also can improve infiltration of the fiber surface. Here, changes in $-C{\overset{O}{\underset{OH}{\diagup\diagdown}}}$ content are even more significant (see Figure 5) since they are basically identical to the laws of change in the ILSS of the previously described composite.
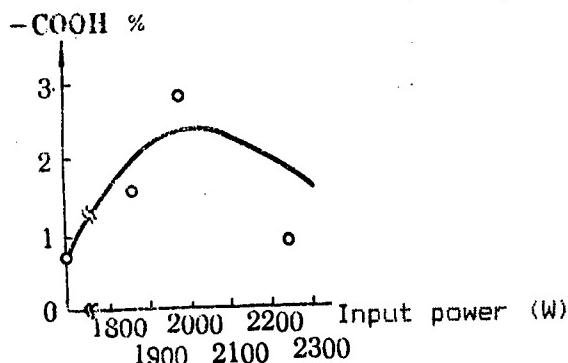

Figure 5. Changes in $-C{\overset{O}{\underset{OH}{\diagup\diagdown}}}$ Content of Carbon Fiber Surface With Input Power

This shows that in these oxy radicals, $-C{\overset{O}{\underset{OH}{\diagup\diagdown}}}$ may make an even greater contribution to increasing composite ILSS. The contribution by $-C{\overset{O}{\underset{OH}{\diagup\diagdown}}}$ may be achieved mainly via these two reactions:

1) Reaction between $-C{\overset{O}{\underset{OH}{\diagup\diagdown}}}$ and the epoxy base causes a chemical crosslink:
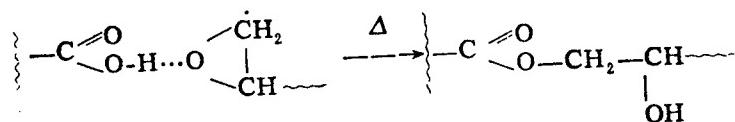
2) Reaction between $-C{\overset{O}{\underset{OH}{\diagup\diagdown}}}$ and amino groups in the resin creates hydrogen bonds:

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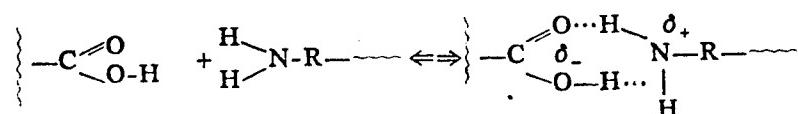
IV. Conclusion

1. There are visibly stronger results from dynamic continuous cold plasma surface treatment of carbon fiber. It can increase the ILSS from 641 kg/cm^2 before treatment to 871 kg/cm^2, attaining the level of Japan's T-300 [fiber].

2. Combined action of three factors increases composite ILSS. First is an increase in the $-C\overset{\nearrow O}{\underset{-OH}{}}$, $>C=O$, and $\geq C-OH$ content of the carbon fiber surface, which increases chemical crosslinking between fiber and matrix, with $-C\overset{\nearrow O}{\underset{-OH}{}}$ playing a slightly greater role. The second is conversion of some graphitic carbon in the carbon fiber surface into amorphous carbon, increasing carbon fiber surface activation. The third is deepening of grooves in the carbon fiber surface, which increases adhesion boundary and mechanical integration effects between the carbon fiber and matrix.

3. Cold plasma continuous treatment of carbon fiber is technically simple and easy to do. There is no problem of waste water, waste gas, or industrial residues, and it can be used in conjunction with carbon fiber production lines.

4. Dynamic continuous cold plasma surface treatment equipment can be used for continuous carbon fiber treatment as well as continuous treatment of other fibers and plastic film, giving it a broad range of prospective applications.

References

1. I.L. Kalnin, Fractures in Composite Materials, Proceedings of USA-USSR Symposium, 1st, pp 373-384, 1978.

2. D. Bellavita, Advanced Composite Materials, pp 357-381, 1978.

3. Eric A. Smith, B.A., editor, Carbon Fibers, 1970.

4. Luo Yifeng [5012 4135 1496], New Trends in Polacryloninitrile Carbon Fibers, TANSU JISHU [Carbon Technologies], No 2, 1985.

5. United States Patent No 3,642,513, 15 February 1972.

6. European Patent Application 0 100 934 2/1984.

7. United States Patent No 3,766,829, 4 December 1973.

8. F. Tuinstra and J.L. Koenig, Raman Spectrum of Graphite, Journal of Chemical Physics, Vol 53 No 3, pp 211-200 [as printed], 1970.

9. He Fu [6320 4395], Carbon Fiber Surface Structure and Surface Treatment, TANSU JISHU, No 1, 1984.

Construction of Vaccinia Virus Containing Hepatitis B Surface Antigen Gene

[Article by Wang Yuan [3076 0997], Zhong Wuwei [6988 2976 1218], Wu Xue [0702 7185], Feng Zongming [7458 1350 6900], Kong Yuying [1313 3768 5391], and Li Ziping [2621 6528 1627], Shanghai Institute of Biochemistry, Chinese Academy of Sciences; and Yan Zilin [0917 1311 2651], National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health: "Construction of Low Toxicity Recombinant Vaccinia Virus GH-1 Containing a Hepatitis B Surface Antigen Gene"]

[Text] Abstract: A safe vaccinia virus of low toxicity, Guang-9, was used as the starter strain for the construction of a recombinant vaccinia virus, GH-1, having the hepatitis B surface antigen (HBsAg) gene (adr sub-type) in the thymidine kinase (TK) gene of vaccinia virus. The GH-1 reproduced in the primary chicken embryo cell, and steadily produced approximately 2 micrograms of HBsAg per ml of culture medium. Neurotropic toxicity experimental results from mouse brains demonstrated the toxicity of the recombinant GH-1 to be similar to that of the safe, low toxicity, starting virus Guang-9. The possibility of clinical use of GH-1 against HBV infection is discussed.

China is an area in which type B hepatitis is epidemic, and type B hepatitis is related to the incidence of primary liver cancer. For this reason, a vaccine effective against HBV is extremely necessary. The hepatitis B surface antigen has already been demonstrated to be able to protect against HBV infection.[1] Type B hepatitis hematogenic vaccine made from HBsAg that had been isolated and purified from the serum of a symptom-free carrier has also been formally used clinically. However, since hematogenic vaccine has its limitations, and since safety problems are involved, it does not make an ideal vaccine for preventive inoculations. In recent years, gene engineering has brought about the expression of type B surface antigen genes in yeast cells[2,3], in cultured mammal cells[4,5], and in vaccinia virus.[6-8] Very great advances have been made in research on second generation type B hepatitis vaccines—genetically engineered vaccines.

Our laboratory used the vaccinia virus WR strain as a starting strain in the construction of recombinant vaccinia virus containing the hepatitis B surface antigen gene that was able to effectively express HBsAg. When this recombinant virus was used to immunize rabbits, it produced HBsAg-specific antibodies. Experimental results demonstrated that recombinant vaccinia virus containing hepatitis B surface antigens can be developed into a vaccine against HBV infection.[8]

The WR strain shows fairly strong neutropic toxicity.[9] For clinical application, we switched to the less virile Guang-9 strain of the vaccinia virus strain, Tiantan, which had been used in China against smallpox, using it as the starter strain. We inserted the hepatitis B surface antigen into the thymidine kinase (TK) gene of the vaccinia virus to construct a weakly toxic recombinant vaccinia virus, GH-1. This article reports the construction of the GH-1, hepatitis B surface antigen expression, and neurotropic toxicity results in mouse brains. The possibility of using this weakly toxic recombinant vaccinia virus as a vaccine for clinical use is also discussed.

Materials and Methods

1. Cells and Viruses. A 10 day old chicken embryo was used to prepare the primary chicken embryo cells (CEC). After removing the chicken embryo's head and innards, they were digested in 0.25 percent tripase to produce a single cell suspension; then primary chicken embryo monolayer cells were grown in a DMEM culture medium containing 10 percent calf serum. Juvenile mouse kidney cells (BHK-21) were cultured in a DMEM culture medium containing 10 percent calf serum. Human TK-deficient cells (Human TK⁻143) were cultured in a DMEM culture medium containing 25 micrograms per ml of 5- bromic uracil deoxyribonucleotide (5-BUdR), and 5 percent fetal calf serum. The vaccinia virus reproduced in BHK-21 cells, or in primary chicken embryo cells, under the conditions described above.[8]

2. Cell Transfection

After the BHK-21 cells were infected by vaccinia virus Guang-9 strain for 2 hours, transfection was done using DNA containing expressed plasmid pJPH-1 that had been precipitated using calcium phosphate.[8,10]

3. Recombinant Virus Screening. Screening of the thymus thymidine kinase-deficient (TK⁻) recombinant virus was done through bacteriophage plaque analysis in the presence of 5-BUdR.[11,12]

4. Virus DNA Extraction and Hybridizing. After the virus infected cells were cleaved using SDS, and processed by protease K, phenol and chloroform were used to remove the protein and extract all of the DNA. Then the dot hybridizing method was used to assay the recombinant virus DNA.[12]

5. Assay of Recombinant Virus Toxicity. After the virus infected cell suspension was frozen and thawed three times, it was diluted with a 2 percent calf serum phosphate buffered saline (PBS) at proper dilution, and the titer was assayed in primary chicken embryo cells. Then 0.03 ml of the virus diluent was injected into the upper part of the brain of Bal b/c white mice weighing between 10 and 12 grams, six mice being injected each time. After observation for 14 consecutive days, the LD_{50} value was calculated.[13]

6. HBsAg Assay. A radioimmunity kit (AUSRIA II, Abbott) was used to assay the amount of HBsAg in the recombinant virus infected cell culture medium and in the cell cleavage medium. The cell cleavage medium was prepared from a cell suspension that had been frozen and thawed three times.

Results of Experiment

1. Construction of Recombinant Vaccinia Virus GH-1

The in vitro recombination method could not be simply applied to the construction of the recombinant vaccinia virus. It was necessary, first of all, to insert a gene of external origin in vitro into a vector containing a vaccinia virus non-essential sequence and a promoter gene to construct an expressing plasmid. Then, internal recombination with the DNA from the starting strain of virus was done.[10] We constructed an expressing plasmid pJPH-1[8] containing a HBsAg as illustrated in Figure 1. Located upstream from the HBsAg gene initiator codon ATC in the pJPH-1 plasmid is the promoter gene $P_{7.5}$ that codes for vaccinia virus 7.5k protein. The HBsAg gene is able to express effectively under regulation of the $P_{7.5}$. On both sides of the HBsAg gene are vaccinia virus TK gene sequences. The TK sequences provide homologous sequences and screening labels for internal recombination.

Two hours following infection with the vaccinia virus strain, Guang-9, the juvenile mouse kidney cells (BHK-21) were transfected with the expressing plasmid pJPH-1, and the cells were harvested 48 hours later. Following vaccinia virus TK homologous sequence internal recombination, the HBsAg gene in the pJPH-1 plasmid was inserted into the vaccinia virus TK gene causing the inactivation of the TK gene; thus, the recombinant virus must be TK-. Figure 1 shows internal recombination. An equal volume of 0.25 percent trypsase was used in the cleavage of the transformed cell medium. After proper dilution, the human TK- 143 cells were transfected, only TK- virus being able to grow bacteriophage plaque in the presence of 5-BUdR, making it easy to screen it out.[10,12] The selected TK- bacteriophage plaque reproduced one generation in human TK- cells in the presence of 5-BUdR. The radio-immune method was used to assay HBsAg expression in the culture medium. We selected a recombinant virus with a fairly high HBsAg expression for the selection and purification of plaque from primary generation chicken embryo cells, finally obtaining the recombinant virus GH-1.
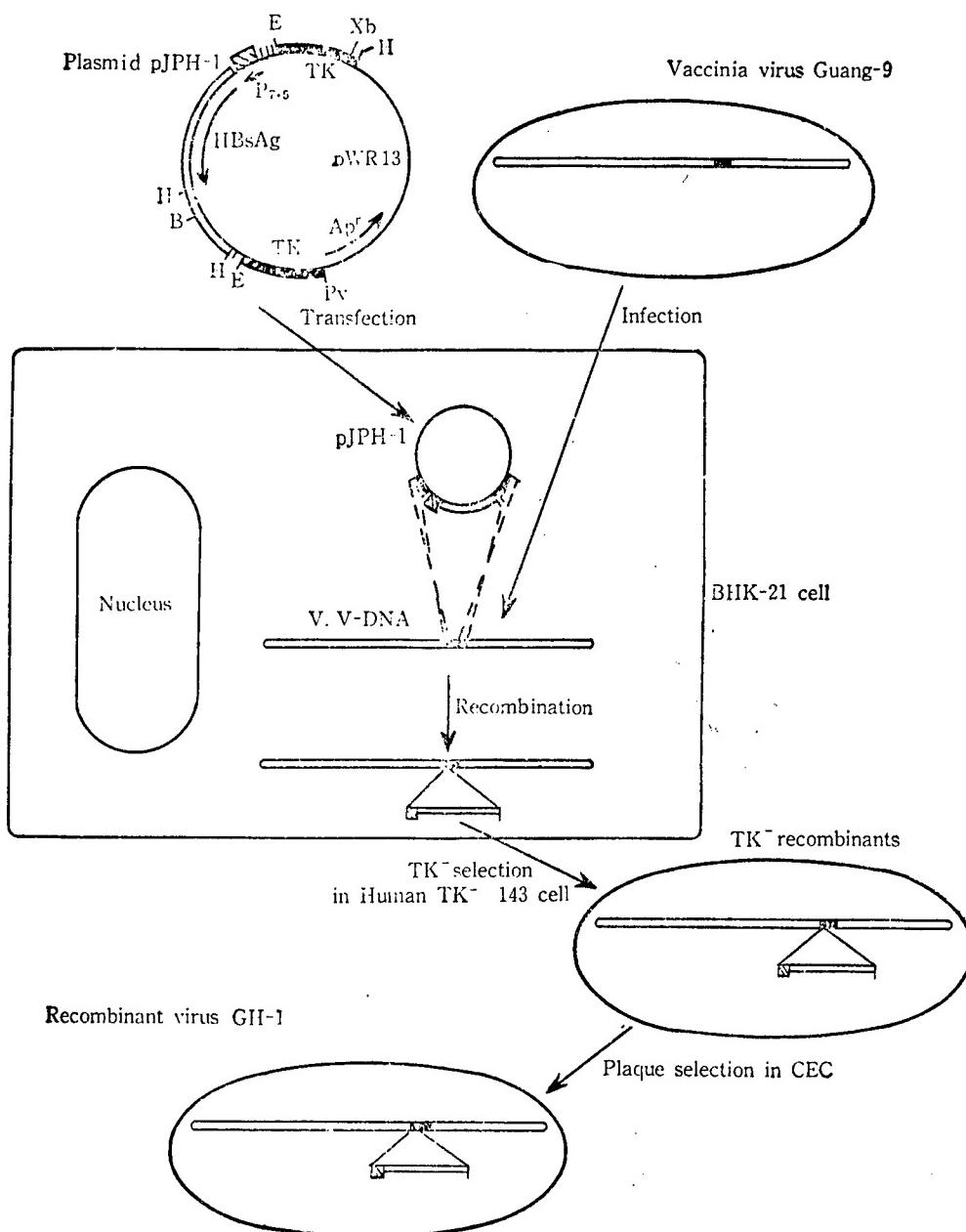
Figure 1. Strategy For the Construction of Recombinant Vaccinia Virus GH-1
Restriction Enzyme: E, EcoRSi: B, BamHI; P, PvuII; H, Hind III; Xb, XbaI
                      CEC: Primary chicken embryo cell

2. Assay of the Recombinant Virus GH-1

We selected DNA from the recombinant virus GH-1, and from the starter virus,
Guang-9 for probing with both HBsAg gene sequences and pWR13DNA, and for
dot hybridizing assay.[12]   The GH-1 virus DNA was able to hybridize with
the HBsAg gene sequence; however, it could not hybridize with the Guang-9

strain DNA (see Figure 2a), showing that the GH-1 genome had been inserted into the HBsAg gene. The GH-1 and the Guang-9 strain DNA were unable to hybridize with the pWR13DNA (Figure 2b). In addition, we used different amounts of the expressing plasmid pJPH-1 DNA as a control, the pJPH-1 additionally containing an HBsAg gene sequence and a pWR13 plasmid sequence; thus, it was able to hybridize both with the HBsAg probe and with the pWR13 probe. When the pWR13DNA is 1 ng, it will still be able to produce hybrid dots. This amount was only one-tenth of the single copy amount of pWR13DNA per microgram of recombinant virus DNA. When the amount of GH-1 DNA reached 5 micrograms per hour, it was no longer able to hybridize with the pWR13DNA probe. Results of the experiment showed there was no insertion of the pWR13 plasmid DNA segment in the GH-1.
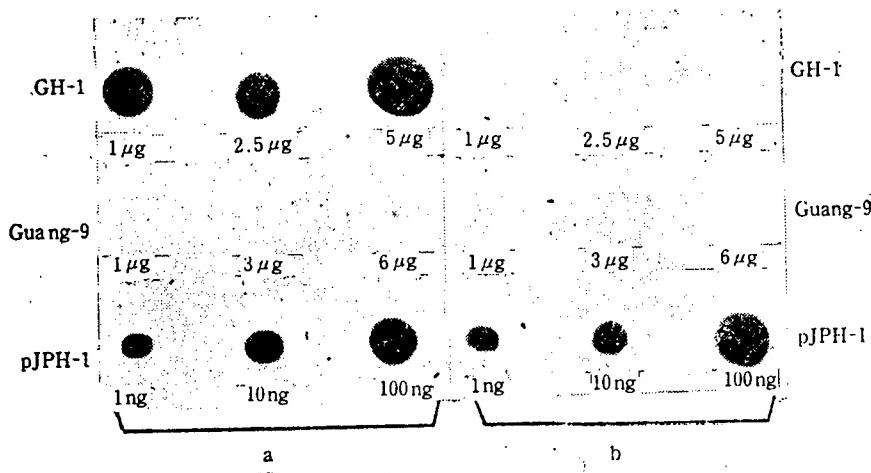

Figure 2. Dot Hybridization of Recombinant Vaccinia Virus GH-1
a, ^{32}P-HBsAg as probe; b, ^{32}P-pWR13 as probe

3. HBsAg Expression

The recombinant vaccinia virus GH-1 was able to effectively express and secrete HBsAg in the tissue cultured cells. We used West German HBsAg positive serum as a quantitative reference standard (Paul Ehrlich Institute, Frankfurt, West Germany), and we used the radio-immune assay method (AUSRIA II, Abbott) to assay GH-1 HBsAg expression. After 1 x 10^6 TK$^-$ 143 cells had been transfected for 24 hours with a 1 x 10^6 PFU (a bacteriophage plaque formation unit) recombinant virus, it produced approximately 2 micrograms of HBsAg, three-fourths of which was dissolved in the cell culture medium. This resembled the recombinant viruses vVH-5[8] and the vVH-14[14] constructed from the WR strain that had been used as a starter strain. Table 1 compares their HBsAg expression.

Table 1. Comparison of the HBsAg Expression of Different Recombinant Viruses

Virus	HBsAg (ng/10^6 cells)	
	Culture Medium	Cell Extract
GH-1	1500	590
vVH-5	2000	440
vVH-14	1500	450
vVH-P1	approx. 1	n.d.
WR	less than 1	less than 1

The HBsAg output per unit of volume was increased in the primary chicken embryo cells that had been GH-1 infected and close cultured. When 1 x 10^7 primary chicken embryo cells were cultured in 5 ml of DMEM containing calf serium, after between 48 and 72 hours of infection with 1 x 10^6 PFU of GH-1 virus, each ml of culture medium yielded approximately 2 micrograms of HBsAg (see Table 2).

Table 2. HBsAg Expression of GH-1 in Primary Chicken Embryo Cells

No.	P/N (1:500)	HBsAg (ng/ml)
1	11	2,750
2	6	1,500
3	12	3,000
4	8	2,000
Average value of HBsAg		2,300

4. Assay of Recombinant Vaccinia Virus Toxicity

We compared the neurotropic toxicity in mouse brains of the GH-1 and the starter strain, GH-9, as well as of the vVH-5 recombinant virus, which we had constructed a long time previously, and its starter strain virus, WR.

We reproduced and tested the titer of the above named recombinant viruses and their starter strain viruses in primary chicken embryo cells. The virus titers were readjusted to 1.4 x 10^8 - 1.5 x 10^8 PFU/ml. After diluting to the proper dilution, they were injected into Bal b/c mice. Test results were as shown in Table 3. Toxicity of the weakly toxic starter strain, Guang-9, and of its derivative GH-1 strain were much lower than for the WR strain and its derivative vVH-5 strain. This demonstrated the weakly toxic Guang-9 strain still to be a weakly toxic strain of the starter strain recombinant virus, GH-1. The toxicity of the GH-1, which had derived from the Guang-9 strain was markedly lower than the vVH-5 strain that had derived from the WR strain. There was a difference of more than 100 times in the number of virus phage plaques needed to be semi-lethal to the mice. Moreover, toxicity of the recombinant viruses were slower than that of the separate starter virus strains, the toxicity of the vVH-5 and the WR strains differing by approximately one order of magnitude. The number of

phage plaques or GH-1 needed to be semi-lethal to the mice was also approximately double the number from the Guang-9 strain. These results were synonymous with those reported by Moss et al for the lowering of vaccinia virus toxicity following TK gene insertion inactivation.[15]

Table 3. Comparison of LD_{50} of Different Recombinant Viruses in Mice

Virus	PFU/ml	LD_{50}	Plaque Number for LD_{50}
WR (China)	1.4×10^8	Equal to or greater than 5.40	Equal to or less than 17
vVH-5	1.4×10^8	4.67	90
Guang-9	1.5×10^8	2.90	5692
GH-1	1.5×10^8	2.67	9621
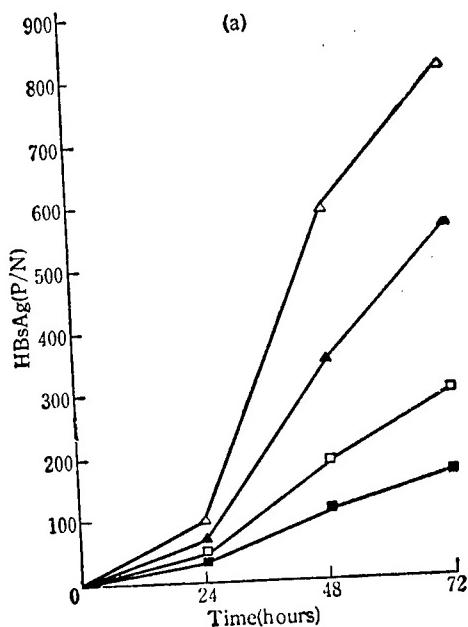
Fig. 3a The effection of cell density on HBsAg expression

■——■, 1×10^6 cells infected with 1×10^6 PFU of GH-1;

□——□, 2×10^6 cells infected with 1×10^6 PFU of GH-1;

▲——▲, 5×10^6 cells infected with 1×10^6 PFU of GH-1;

△——△, 1×10^7 cells infected with 1×10^6 PFU of GH-1
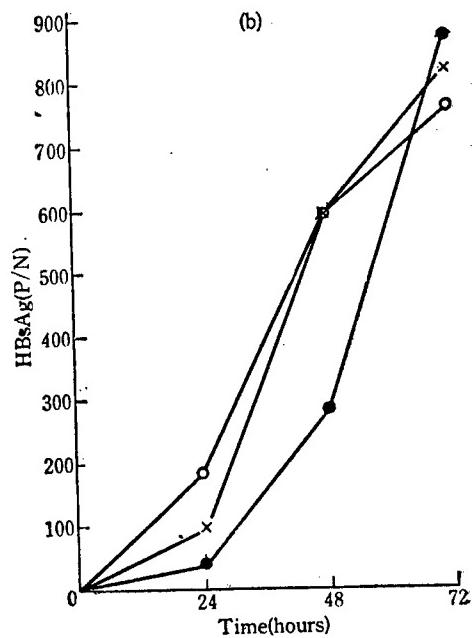
Fig. 3b The HBsAg expression in CEC infected with different amount of GH-1

●——●, 1×10^7 cells infected with 1×10^5 PFU of GH-1;

×——×, 1×10^7 cells infected with 1×10^6 PFU of GH-1;

○——○, 1×10^7 cells infected wtih 1×10^7 PFU of GH-1

CEC: primary chicken embryo cell

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We also made a comparison of the effect of GH-1 and vVH-5 recombinant vaccinia viruses as well as their derivative strains, Guang-9 and WR, when injected intracutaneously into gray rabbits. Measurement of the diameter of the skin infiltration on the fourth day showed the skin reaction from both the Guang-9 and the GH-1 as less than that from the WR and vVH-5 strains. This was consistent with the results obtained from the mouse brain toxicity assay.

5. Culturing Conditions and Their Effect on GH-1 Expression

We compared the expression of GH-1 in primary chicken embryo cells under different culturing conditions. First we used the same quantity of virus (1×10^6 PFU) to infect different quantities of chicken embryo cells as shown in Figure 3a. The HBsAg diluted in the culture medium increased as the concentration of chicken embryo cells increased. Second, we determined the correct proportion of virus to cells for GH-1 infection of primary chicken embryo cells. The curved line in Figure 3b shows a comparison of the amount of HBsAg expression when 1×10^7 primary chicken embryo cells were infected with different amounts of GH-1 virus. The results showed that when the amount of inoculated virus was small, the HBsAg content of the culture medium was fairly low on the first day following cell infection; however, HBsAg output on the third day following infection was fairly high.

As an aid in the isolation of HBsAg, preliminary observations were made of GH-1 expression in serum-free culture medium. 1×10^7 primary chicken embryo cells were infected with 1×10^6 PFU of GH-1 in 5 ml of culture medium. Figure 4 shows the time curve for HBsAg expression after GH-1 infection of chicken embryo cells using a culture medium containing 10 percent calf serum. Following infection by GH-1, the calf serum culture medium was kept warm for 1 day after which it was divided into two parts. The first part continued to be maintained by a culture medium containing 10 percent calf serum. The other part was changed to a serum-free culture medium. Results of the experiment demonstrated that after use of a serum-free culture medium, the GH-2 continued the effective expression of HBsAg, the quantity of expression being approximately two-thirds that when the 10 percent calf serum culture medium was used. If the culture medium was changed two or three times in the course of culturing in a serum-free culture medium, the HBsAg output was about the same as from the single use of a culture medium containing 10 percent calf serum.
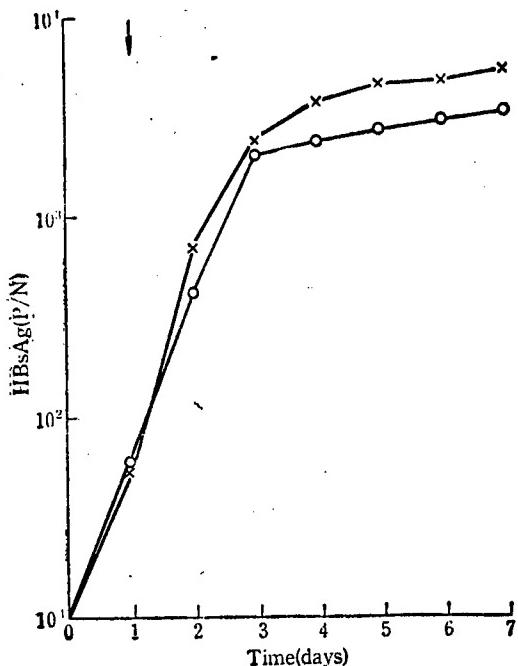
Figure 4. Time Curve for HBsAg Expression of GH-1 i Primary Chicken Embryo Cells

x - x, Culture medium containing 10 percent calf serum
o - o, Culture medium without calf serum

Discussion

Vaccinia virus used as a live vaccine made a tremendous contribution to mankind in wiping out smallpox through the world. Its effectiveness and safety has been fully tested through much clinical use over a long period of time. In 1983, Smith et al. used vaccinia virus as a vector in the successful expression of hepatitis B surface antigen genes, pioneering a new road in the research and development of genetically engineered vaccines.

We inserted hepatitis B surface antigen genes into the vaccinia virus genome to construct a recombinant vaccinia virus containing a hepatitis B surface antigen gene. Results of the experiment demonstrated that hepatitis B recombinant vaccinia virus GH-1 still had the infectiousness and the broad host range of the original virus, and it was also able to consistently produce and release hepatitis B surface antigens. Thus, it could serve as a live virus in direct clinical application, and it could also be used to prepare hepatitis B sub-unit vaccine.

1. Low Toxicity and Safety of GH-1

A recombinant vaccinia virus for use in a live vaccine must have low toxicity and be safe. GH-1 was constructed by using the weak toxic vaccinia virus, Guang-9, as the starter strain. It, like the vVH-5 recombinant virus that was constructed by using the WR strain as a starter strain, can

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effectively express and secrete HBsAg granules; however, toxicity has declined markedly. Comparison of neurotropic toxicity in mouse brains demonstrated a more than 100-fold difference between the two in toxicity. (See Table 3)

The TK gene that we used in the expressed plasmid pJPH-1 in the GH-1 construction process came from the WR strain; however, the toxicity of the recombinant virus GH-1 obtained from the recombination of that plasmid with the Guang-9 strain was markedly lower than that of the WR strain, and similar to that of the Guang-9 strain. This demonstrated that the vaccinia virus' TK sequence is not a gene segment that plays a decisive role in the toxicity of the vaccinia virus WR strain, and it had no marked effect on the toxicity of the Guang-9 strain. Results of a comparison of part of the nucleotide sequence of the TK gene in the Guang-9 and WR strains showed no specific base mutant location.[16] Additionally, this was consistent with the report of Moss et al. After a gene of external origin is inserted into the vaccinia virus TK gene, it serves to reduce the toxicity of the vaccinia virus.

A descent cell, the human TK⁻ 143 cell, was used in the construction of the GH-1; however, numerous plaque selections and purifications of the recombinant virus in the primary chicken embryo cells should be able to remove the unsafeness caused by the descent cells.

In addition, the outcome of the dot hybridization of some of the pWR13DNA of the expressed plasmid vector used as a probe with the GH-1 DNA demonstrated (Figure 2) that there was no insertion into any other DNA segment except the HBsAg gene segment in the GH-1 DNA. In short, the above stated GH-1 is of low toxicity and safe, and holds the possibility of being used clinically.

Recently there have been reports about results obtained from recombinant vaccinia virus in the immunization of humans. After Jones accidentally inoculated a recombinant vaccinia virus into which a cystic stomatitis virus (VSV) glucoprotein had been inserted,[17] the serum produced a specific anti VSV glucoprotein antibody, but no other adverse reaction occurred. The starter strain of vaccinia virus was the WR strain. Had a weakly toxic strain been used, it would have been even safer.

2. GH-1 and a Sub-Unit Vaccine Producing System

Use of vaccinia virus as a vector to express hepatitis B surface antigen genes has yet another characteristic, namely, that once the hepatitis B recombinant vaccinia virus infects cells, there is no difference between the secretion and the glycosidifaction of the surface antigens produced and the surface antigen plasmids existing in the patient's serum. Therefore, this provides a fairly ideal new way in which to purify surface antigens and prepare genetically engineered sub-unit vaccine. In comparison with the yeast system, since the surface antigen products are glycosided and secreted, their immunogenicity may be better than that of the yeast system, and simpler and more economic to isolate and purify than the yeast

system as well. In comparison with the mammalian cell system, descent cell culturing may be used, avoiding conflict with the descent cells that are used and being safer. It would also get around the difficulty of large scale continuous cell propagation and culturing. In addition, reliable experience has been gained over a long period of time in the culturing of vaccinia viruses, so they can be produced and spread in large quantities. This is also a conspicuous advantage.

We have preliminarily studied conditions for the use of primary chicken embryo cell in culturing GH-1, finding that HBsAg output may amount to as much as approximately 2 micrograms per ml. Moreover, expression remains steady from one generation to another. After transmittal through 16 generations, HBsAg output remained at the 1 micrograms per ml level (Data not shown).

We collected the primary chicken embryo cell culture medium that had been GH-1 infected, and purified it using single anti-affinity column chromatography or ion exchange. This initially purified HBsAg was used to immunize mice, which rapidly and effectively produced anti-HBsAg specific antibodies. LD_{50} testing also demonstrated their antigenicity to be similar to that of serum vaccine.[18] Further purification work is underway.

REFERENCES

1. Tiollias, P., et al. Biology of Hepatitis B Virus, Science, 1981, 213, 406.

2. Valenzuela, P., et al. Synthesis and Assembly of Hepatitis B Virus Surface Antigen Particle in Yeast. Nature, 1982, 298, 347.

3. Shen Luping [3088 4845 5493] et al. Hepatitis B Virus HBsAg Gene Expression Under Gal-10 Control in Yeast. SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1984, 16, 320.

4. Crowley, C.W., et al. Plasmic Directed Synthesis of Hepatitis B Surface Antigen in Monkey Cell. Mol. Cell. Biol., 1983, 3, 44.

5. Wang, Y. et al. Enhanced Production of Hepatitis B Surface Antigen in NIN 3T3 Mouse Fibroblasts By Using Extrachromosomally Replicating Bovine Papilloma Virus Vector, ibid., 1983, 3, 1032.

6. Smith, G.L., et al. Infectious Vaccinia Virus Recombinants That Express Hepatitis B Virus Surface Antigen. Nature, 1983, 302, 490.

7. Paoletti, E., et al. Construction of Live Vaccines Using Genetically Engineered Pox Viruses: Biological Activity of Vaccinia Virus Recombinants Expressing the Hepatitis B Virus Surface Antigen and Herpes Simplex Virus Glycoprotein D. Proc. Nat. Acad. Sci. USA., 1984, 81, 93.

8. Wang Yuan et al. Recombinant Vaccinia Virus Carrying a Hepatitis B Surface Antigen Gene--A Possible Live Vaccine Against Hepatitis B. ZHONGGUO KEXUE (B) JI [CHINESE SCIENCE (B) VOLUME], 1986, 4, 393.

9. Mark, R., et al. Genetic Basis For Vaccinia Virus Virulence. Vaccinia Viruses as Vectors For Vaccine Antigens, 1985, 37, Quianan, G.V., Jr., (ed).

10. Weir, R., et al. Mapping of the Vaccinia Virus Thymidine Kinase Gene By Marker Rescue and Cell Free Translation of Selected mRNA. Proc. Nat. Acad. Sci. USA., 1982, 79, 1210.

11. Mackett, M. et al. Vaccinia Virus Recombinants: Expression of VSV Genes and Protective Immunization of Mice and Cattle. Science, 1985, 227, 443.

12. Mackett, M., et al. Vaccinia Virus: A Selectable Eukaryotic Cloning and Expression Vector. Proc. Nat. Acad. Sci. USA., 1982, 79, 7415.

13. Beijing Xiehe Hospital Testing Section: Virus Experimental Testing Handbook. People's Health Press, 1960, 25.

14. Wang Yuan et al. Hepatitis B Surface Antigen Gene Expression Using Vaccinia Virus As a Vector. [SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1984, 16, 698.

15. Moss, B., et al. Live Recombinant Vaccinia Virus Protects Chimpanzees Against Hepatitis B. Nature, 1984, 311, 67.

16. Wu Xue et al. Construction of pGJP-5, A Plasmic Commonly Used with Vaccinia Virus. [SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1987, 19, 397.

17. Jones, L, et al., Accidental Human Vaccination With Vaccinia Virus Expressing Nucleoprotein Gene, Nature, 1986, 319, 543.

18. Chen Zhenzhen [7115 3791 3791]: Immunogenicity of HBsAg Produced by Recombinant Vaccinia Virus. [SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1986, 18, 534.

Pattern Recognition Technique For Classifying Scorpion Toxins Explained

[Article by Wang Peng [3769 7720] and Zhou Xinhua [0719 2450 5478], Shenyang College of Pharmacy: "Application of Pattern Recognition to the Classification of the Primary Structure of Scorpion Toxins"]

[Text] Abstract: This article reports the application of pattern recognition to the classification of 11 scorpion toxins, the primary structures of which have been previously determined, reaching the same conclusion obtained by the serum immunological method. The method was applied to determine how a recently assayed scorpion venom, Bmk III, should be classified.

Scorpion mammalian toxins possess important physiological functions that are increasingly the serious attention of scientists. Foreign study of scorpion toxins has moved with extreme rapidity in recent years. As of the moment, approximately more than 50 different scorpion mammalian toxins have been isolated from scorpion venom and purified, and the amino acid sequences of all or most of them have been assayed.[1] Today they are a tool that is indispensable to the study of sodium-potassium passage through excitable membranes, and they have been used as a basis for explaining the development and evolution of scorpion species. The functioning of proteins and peptides is determined by their higher structures, and the formation of higher structures relies, to a very large extent, on primary structures, namely amino acid sequences. Obviously, an analysis of the primary structure of scorpion toxin will not only be able to explain functional differences among them, but will also be able to explain the interrelationship among them. This is the only way to quantify the classification of scorpion species. In 1977, Possani, a Mexican, used mathematical statistics in the analysis of five types of scorpion toxins for which the N-terminal amino sequence was already known that was based on the extent to which the amino acid sequences of individual toxins were the same in size.[2] Subsequently, Rochat et al classified scorpion mammalian toxins into five categories in terms of consistency of all amino acid sequences and their immunity interaction with blood serum.[3] Use of artificial methods to classify the primary structures of proteins is a time consuming and arduous task.

As computer techniques have developed, pattern recognition as an effective way of classifying and recognizing has become widely applied to all areas. Therefore, we have tried pattern recognition in the classification of scorpion toxin primary structure, and compared the findings with those obtained from classification using serum immunology with gratifying results.

Scorpion Toxins and Selection of Their Characteristics

Eleven scorpion toxins for which the amino acid sequence had already been determined were selected as initial specimens for classification, and individual amino acids in the primary structure of the scorpion toxins were then selected for classification. For convenience in comparing differences among scorpion toxins, cysteines, "C," in the primary structures were individually brought into register at the time of input, and when of insufficient length, a "-" was inserted to make up. In this way, the 11 scorpion toxins had 77 characteristics, and these characteristics made up 77 spaces. The multidimensional distance of each of the scorpion toxins was used as the scorpion toxin classification standard, i.e., the doubles in their primary structure were compared. If they differed for corresponding amino acids, the distance was increased by one; otherwise no increase was made. Classification and recognition of scorpion toxins was done in this way. Table 1 shows the ranking of amino acid sequences for the 11 scorpion toxins.

Table 1. Eleven Scorpion Toxins and Their Characteristics

No	Name		Characteristics
1	AaH	I	-KRDGYIVYPN-NCVYHCVPP-----CDGLCKKN-GGSSGSSC -FLVPSGLACWC-KDLP-DNVPIKDTSRK--CT-
2	AaH	I'	-KRDGYIVYPN-NCVYHCIPP-----CDGLCKKN-GGSSGSSC -FLVPSGLACWC-KDLP-DNVPIKDTSRK--CT-
3	AaH	I"	-KRDGYIVYPN-NCVYHCVPP------CDGLCKKN-GGSSGSSC -FLVPSGLACWC-KDLP-DNVPIKDTSRK--CTR
4	AaH	III	-VRDGYIVNSK-NCVYHCVPP-----CDGLCKKN-GAKSGS -CGFLIPSGLACWC-VALP-DNVPIKDPSYK--CHS
5	AaH	II	-VKDGYIVDDV-NCTYFCGR---NAYCNEECTKL-KGESG -YCQWASPYGNACYCYK-LP-DHVRTKGPGR---CH-
6	Lqq	V	-LKDGYIVDDK-NCTFFCGR---NAYCNDECKKK-GGESG -YCQWASPYGNACWCYK-LP-DRVSIKEKGR---CN-
7	Amm	V	-LKDGYIIDDL-NCTFFCGR---NAYCDDECKKK-GGESG -YCQWASPYGNACWCYK-LP-DRVSIKEKGR---CN-
8	Bot	II	-GRDAYIAQPE-NCVYECAK---NSYCNDLCTKN-GAKSG -YCQWLGRWGNACYC-IDLP-DKVPIRIEG-K--CHF
9	Bot	I	-GRDAYIAQPE-NCVYECAQ---NSYCNDLCTKN-GATSG -YCQWLGKYGNACWC-KDLP-DNVPIRIPG-K--CHF
10	Css	II	--KEGYLVSKSTGCKYECLKLGDNDYCLRECKQQYGKSSGG YCYAF-----ACWC-THLYEQAVVWPLPN-KT-CN-
11	Lqq	IV	GVRDAYIADDK-NCVYTCGS---NSYCNTECTKD-GAESG -YCQWLGKYGNACWCJK-LP-DKVPIRIPG-K--CR-

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Classification of Scorpion Toxins

First a non-linear mapping method of scorpion toxin classification was used for pattern recognition,[4] meaning, that in a situation of little loss of information about the relative location among the classified to be classified, every effort was made to display the data on a flat surface from a high number of dimensional spaces to two dimensional spaces. This was a convenience in the evaluation through direct observation of the mapping results, and in ascertaining and recognizing the structure of spatial data points. In addition, the discontinuity of data points permitted a natural definition of point clusters.

If the number of specimens is N, and the number of characteristics is M, the result function is as follows:

$$K = \frac{1}{\sum\limits_{p<q}^{N} d_{pq}^*} \sum\limits_{p<q}^{N} \frac{(d_{pq}^* - d_{pq})^2}{d_{pq}^*} \quad (1)$$

the two dimensional distance between d_{pq} specimen p and specimen q
the distance between d^*_{pq} specimen p and specimen Q in M dimensional space
Constant readjustment of the location of specimens p and q in two dimensional space produced the following with regard to all specimen points:

$$K^* = \min(K)$$

This showed that the relative location in two dimensional space of all specimens at this time was most able to represent their relative positions in M dimensional space.

We worked out this calculation method using an Apple II computer. Table 2 shows the coordinates and the result function values for the 11 scorpion toxins after mapping in two dimensions. Figure 1 is a non-linear map drawn on the basis of the Table 2 data.

Table 2. Mapping Coordinates of 11 Scorpion Toxins and the Value of Function

| Sample	First coordinate	Second coordinate
1	−9.85	−17.66
2	−9.45	−16.77
3	−10.94	−17.22
4	−18.63	−12.76
5	13.62	15.11
6	9.74	12.48
7	9.90	9.61
8	−11.56	19.61
9	−9.50	14.93
10	32.51	−22.01
11	−22.00	10.02
Value of function	0.118	
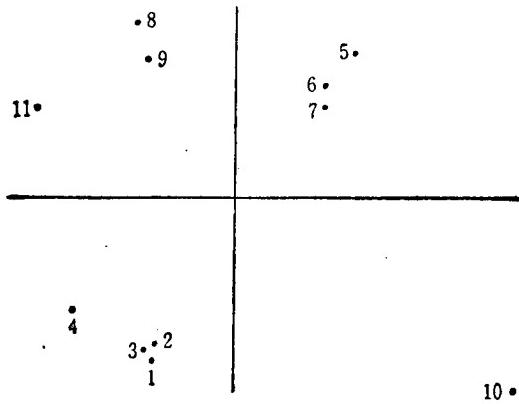
Fig. 1   Non-linear mapping of 11 scorpion toxins

It can be seen that the scorpion toxins have been clearly divided into five groups.   Scorpion toxin numbers 1,2,3, and 4 are in one group (designated Group I); scorpion toxin numbers 5, 6, and 7 are in another group (designated Group II); scorpion toxin numbers 8 and 9 are in yet another group (designated Group IV; and scorpion toxin numbers 10 and 11 are each in a category of their own (designated Group V and Group III). In 1981, Rochat and Delori used an anti-serum immunological method produced a classification whereby number 1 AaH I and number 4 AaH III form a unit; number 5 AaH II, number 6 Lqq V and number 7 Amm V form a unit, and number 8 Bot II and number 9 Bot I form yet another unit. Classification results obtained were the same from the pattern recognition method and the serum immunology method. (See Table 3)

Table 3.   Classification Results Using the Pattern Recognition and the Serum Immunology Methods

Group	Structural standard	Immunological standard
1	AaH I, AaH I', AaH I'', AaH III	AaH, I, AaH III
2	AaH II, Lqq V, Amm V	AaH II,Lqq V,Amm V,Bot III,Bot XI
3	Lqq IV	
4	Bot II, Bot I	Bot I, Bot II, Bot IV, Bot V, Bot VII. Bot VIII, Bot IX, Bot X
5	Oss II	

Recognition of Unclassified Scorpion Toxins

After having divided the aforestated 11 scorpion toxins into five groups, the toxins in each group may be used as a pattern for recognizing in which group new scorpion toxins belong.   Classification of new scorpion toxins follows the principal of greatest subordination.   Formula (2)1 may be used to calculate the degree of subordination of each category pattern,[5] new scorpion toxins belonging to the group having the greatest degree of subordination.

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$$u_{ik} = \frac{1}{\sum\limits_{j=1}^{c}\left(\dfrac{d_i}{d_j}\right)^2}$$ (2)

U_{ik} scorpion toxin k subordination to the i group.
c number of classifications

average distance of d_i and d_j scorpion toxins from the i group and the
j group pattern

We made a classification recognition of the primary structure scorpion
toxin Bmk III[6] most recently assayed in China. Its primary structure was:

VRDAYIAKPENCVYECATNEYCNKLCTDNGAESGYCQWVGRYGNACY/
WCIKLPDRVPIRVWGKCHG

The locations of all but 47 amino acid have been determined, and for those
47, X was substituted. Since formula (2) calculated their degree of
subordination to groups I through V as being 0.09, 0.12, 0.30, 0.45, and
0.04, they should belong to Group IV according to the principle of greatest
subordination. This is consistent with results of the analysis made by
Liu Jianning [0491 1696 1337] et al., i.e., it has fairly great affinity
with the Sudanese Buthus occitanus tunetanus nerve toxins I and II.

Conclusions

Study of scorpion toxins is still continuing, with new scorpion toxins
constantly being isolated and assayed. The work reported in this article
has used pattern recognition in the classification of scorpion toxins for
which the structure was previously determined, and it identified the
classification of new scorpion toxins, producing results identical with
those obtained from the use of serum immunology, the two of which corroborate
each other.

REFERENCES

1. Zhou Xinhua: Biochemical Study and Clinical Application of Scorpion
   Venom. SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et
   BIOPHYSICA SINICA], 1984(2), 20.

2. Possani, L.D. Purification and Properties of Mammalian Toxins From
   the Venom of the Brazilian Scorpion Tityus Sorrulatus Lutz and Mello,
   Archs. Biochem. Biophys., 1977, 180, 394.

3. Rochat, H., Bernard, P., and Courand, F. Scorpion Toxins: Chemistry
   and Mode of Action, Advance in Cytopharmacology, 1979, 3, 325.

4. Li Jiegu [2621 0094 6253] and Cai Guolian [5591 0948 1670]: Computer
   Pattern Recognition Techniques, 1986, First Edition, 207, Shanghai
   Communications University Press, Shanghai.

5. Lou Shibo [2869 0013 0590], Sun Zhang [1327 4545], and Chen Huacheng [7115 0553 2052]: Fuzzy Mathematics, 1983, First Edition, 122, Science Press, Beijing.

6. Liu Jianning, Yang Duan [2799 3843], and Lei Kejian [7191 0344 0256]: Study of the Primary Structure of Scorpion Venom Nerve Toxin, SHENGWUHUAXUE YU SHENGWUWULI JINZHAN [ADVANCES IN BIOCHEMISTRY AND BIOPHYSICS], 1987 (2), 44.

Hepatitis B PreS$_2$ Surface Antigen Gene Expression in Yeast

[Article by Shen Luping [3088 4845 5493], Yang Zhiyong [2799 1807 0516], Xu Ling [1776 3781], Xie Weijun [6200 0251 6511], Zhu Shengzu [2612 4939 4371], Li Zaiping [2621 6528 1627], Shanghai Institute of Biochemistry, Academia Sinica: "Expression of the Hepatitis B Pre-S$_2$ Surface Antigen Gene in Yeast"]

[Excerpts] Abstract: An expression vector for the HBV pre-S surface antigen (HBpresAg) in yeast was constructed whose expression level under control of the yeast glyceraldehyde-3' dehydrogenase phosphate (GAP) promoter was about 300μ g/l of HBpresAg. Electron microscope observation showed the polypeptides produced to be granular and having a CsCl buoyant density similar to that of the HBsAg produced by yeast. The HBpresAg particles were also able to bond with polymerized human albumin, and to cause the production of specific antibodies in Balb/c mice.

Clinical experiments have demonstrated surface antigen protein vaccine isolated from the blood serum of carriers of the hepatitis B virus to possess immunologic properties, and to be able to block HBV infection effectively. Accompanying advances in genetic engineering has been the production through genetic engineering methods of surface antigen vaccines possessing the same ability to immunize as blood vaccines. Furthermore, the economy and safety of these genetically engineered vaccines has resulted in their gradual replacement of blood vaccines. One effective way of producing genetically engineered vaccines is through the expression of HBV surface antigen proteins by brewer's yeast.[2,3] In 1986, the United States Food and Drug Administration approved the sale for clinical use of hepatitis B vaccine made from yeast.

We applied the ability of the yeast GAP promoter to express HBsAg to the assembly of an expression plasmid vector for the HBpreSAg. The HBpreSAg protein expressed by the yeast was able to bond with polymerized human albumin, was granular, had a CsCl buoyant density similar to that of HBsAg produced by yeast, and possessed specific antigenicity to confer immunity.

Materials and Methods

1. Bacteria Strains and Enzymes Used in the Experiment

Yeast host cells HR 125-5 Dα, HR 125-5Da (MATα or a, trp1^a, leu2$^=$, ura3-52, his3, his4, and rme) were a gift from Professor Herskowitz; DBY 745 (MATα, HMLα, NMRa, ade1-100, leu2-3, leu2-113, ura3-52, and MAL 2 were a gift from Professor Hamer. E. coli JM-83 was the host cell that had been amplified by a recombinant shuttle plasmid.

Plasmid pSII-3 was assembled by Comrade Feng Zongming [4458 1350 6900] of the Shanghai Institute of Biochemistry.

Restriction endonucleases EcoRI, HindIII, PstI, XhoI, and XbaI, as well as DNA polymerase, and T$_4$DNA ligase were provided by the tool enzyme preparation group of the Shanghai Institute of Biochemistry. The SauI was a product of BRL Company.

2. Yeast Transformation and Culturing Conditions

Yeast transformation was done using the Ito method,[11] a transformed strain being screened from a Leu$^-$ synthesized medium, Leu$^+$ colonies being selected.

The transformed bacteria strain was inoculated into 5 ml of YPD medium (2 percent glucose, 1 percent yeast extract, and 2 percent peptone), which was then cultured at 30 degrees C overnight. The next day, this 5 ml was inoculated into 250 ml of the same medium, which was cultured while being agitated at 30 degrees C for between 12 and 48 hours (the length of time varying with requirements of experiments). Every 12 hours, the medium was replenished with 2 percent glucose and the other ingredients. The thalli were collected and a radioimmunoassay (RIA) for HBsAg was done (using either the Abbott AUSRIA kit or the ELISA enzyme marker assay[12]).

3. Assay of Surface Antigen Polymerized Human Blood Serum Albumin Receptor Activity

After cells from 100 ml of the above bacteria solution were collected through centrifuging and crushed,[13] 3 ml of supernate was obtained. Assay of polymerized human blood serum albumin receptor activity was done using the method of Chen Huiying [7115 1979 5391] et al,[14] and an enzyme colorimeter with a 493 nm wave length was used to read out optical density (OD$_{493}$). The number of OD specimens per number of negative control OD was equal to or greater than 2.5; thus positive.

4. Assay of CsCl Density of Polypeptides Expressed by the Yeast, and Assay of Immunization Capability

After the aforementioned recombinant saccharomycetes had been cultured for 48 hours, the thalli were collected and centrifuged at 16,000 rpm. Cell debris was then removed and the fluid condensed. Then 1.1 ~ 1.4 CsCl g/ml

was non-continuously gradient centrifuged at 3,000 rpm for 12 hours. A 0.5 ml tube was used to collect different components, each component being assayed for the amount of surface antigens and CsCl density.

Ammonium sulfate settling was used to purify the above supernate, and dialysis was done. Between 8 and 10 week old Balb/c mice were injected with 0.25 and 0.5 micrograms of surface antigen protein, and the same amounts of surface antigens made from blood were injected into Balb/c mice as a positive control. Physiological saline was injected as a negative control. Three weeks later, blood samples were taken for assay of antibody titer (Abbott AUSAB kit).

Results

1. Construction of an Expression Plasmid Vector For the HBV Pre-S Antigen (HBpreSAg)

According to the HBV (adr sub-type) DNA sequence,[16] an SauI enzyme cutting site is located at 7bp, which is upstream from the $preS_2$ initial code ATG. The plasmic pSII-3 is the DNA segment obtained from the enzymolysis of the HBV genome by SauI-BamHI that has been cloned to a plasmid containing the TK gene and vaccinia virus $p_{7.5}$ promoters, multiple junction points existing for reannealing.[15] After we used SauI to enzymolyze the pSII-3 plasmid to obtain filiform DNA, we used DNA polymerase to make the end sticky and connected it to EcoRI to obtain an HBpresAg gene segment with an EcoRI sticky end. This DNA segment not only contained a complete $preS_2$ region (165 bp), and a surface antigen gene, but it also had a 5' end 7 bp no translation region, and a surface antigen gene 3' end no translation region 565 bp.

Plasmid pGHs-16 was an E. coli pBR 322 plasmid from which the EcoRI site had been eliminated that carried a yeast GAP promoter.[3] Downstream from the GAP promoter a DNA segment of external origin was inserted at the single EcoRI site. The above HBpresAg gene-containing DNA segment was connected at the EcoRI cutting point to the GAP promoter downstream, and recombinant clone strains selected on the basis of plasmid size.

Since there is a cutting point at XhoI in the surface antigen gene $preS_2$ region, and since there is a cutting point at PstI on the DNA of the pGH_3-16 plasmid, use of the PstI and XhoI double enzymolysis plates permitted the conclusion that the direction of insertion of the recombinant HBpresAg gene was identical with the transcription direction of the promoter. As Figure 1 (a) shows, the two DNA strands produced following PstI and XhoI enzymolysis were 2.2 kb and 4.5 kb long; thus, the direction of insertion of the HBpresAg gene was the same as the transcription direction of the GAP. Otherwise, DNA strands measuring 1.6 kb and 5.1 kb would have been produced. The recombinant plasmid pGH_316-SII was the needed recombinant clone strain. For the DNA sequence at the yeast GAP promoter and the $preS_2$ junction site, please see Figure 1 (b).
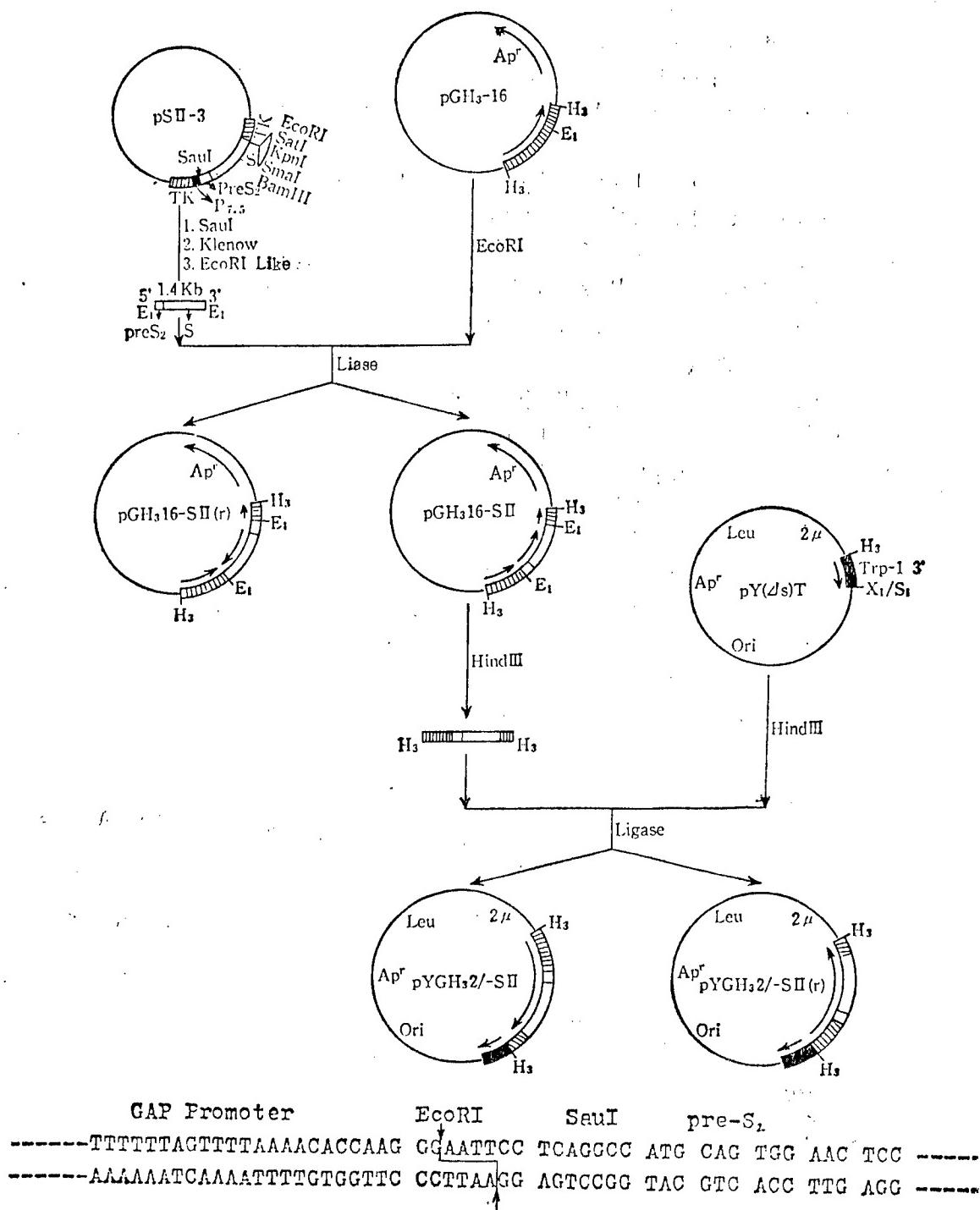
Figure 1. (a) Construction of Yeast Expression Vector for HBpresAg;
(b) DNA Sequence of the GAP Promoter and Pre-S$_2$ Region

In order for the HBpresAg gene to express in the yeast cells, we had to use HindIII enzymolysis to connect the GAP promoter-HBpresAg gene recombinant DNA segment to the E. coli-yeast shuttle plasmid pY (/\s)T^3. In addition, the downstream end of the HBpresAg gene was added to the Trp-1 3' end transcription terminal code in order to improve gene transcription efficiency.

The recombinant plasmid that we constructed was an E. coli-yeast shuttle plasmid containing an HBV HBpresAg gene in which the GAP promoter and its transcription direction were the same. In addition, at the downstream end of this gene was a GAP gene 3' end no translation region 120 bp and a Trp-1 gene 3' end 650 bp containing an effective transcription terminal code no translation region. The expression plasmid was transferred to the different yeast host cells DBY 745, HR125-5Da, and HR 125-5D. The leu$^+$ colonies were screened out and the vitality of the HBsAg that they produced was assayed.

2. HBpresAg Gene Expression and Identification of Polypeptide Products

After the HBpresAg gene-expressed plasmid pYGH$_3$21-SII was transferred into different yeast host cells, there was a very great difference in the level of expression under identical culturing conditions. Expression was highest in the DBY 745 cells (See Table 1), the output from a shaken flask amounting to as much as 300μg/L under proper culturing conditions.

Table 1. Expression Level of pYGH$_3$21-SII in Different Yeast Strains

HBsAg	Yeast strain		
	DBY-745	HR125-5Da	HR125-5Dα
CPM*	2692	369	478
μg/L	110	2.7	8.8

The recombinant yeast cultures were incubated at 30 degrees C for 36 hours.

*After breaking the yeast cells from 50 ml culture, 3.5 mil of supernate was obtained, and then diluted 500 folos for RIA assay (Abbott)

The products expressed by the HBpresAg genes showed up under an electron microscope as having a granular form similar to that of human blood serum HBsAg or yeast HBsAg. After purification of the expressed product, CsCl density gradient centrifuging was done. The vitality peak of the HBpresAg and the HBsAg location produced by the yeast were the same (Figure 2).

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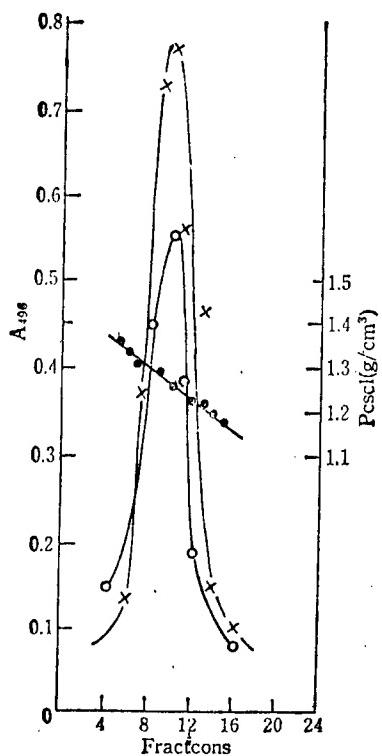
Figure 2. The CsCl Density of HbsAg and HBpresAg Derived From Yeast

CsCl gradient centrifuging of HBsAg (o-o), and HBpresAg (x-x) derived from yeast

The HbpresAg protein isolated from human blood was able to bond with polymerized human blood albumin. Persin et al used the DNA reading frame variant method to change the $preS_2$ regions, but the cells still produced HBsAg protein granules; however, they lost their ability to bond with polymerized human blood albumin. We placed the HBpresAg gene and the HBsAg gene under GAP promoter control, and ran separate assays on the binding to polymerized human blood albumin receptors of the products expressed by the constructed expression plasmids $pYGH_3$ 21-SII and $pYGH_3$ 16r-S. The results (Table 2) show that the HBpresAg produced was able to bind effecitvely with polymerized human blood albumin, but the HBsAg did not. The results of this experiment showed not only that the HBpresAg protein produced by the saccharomycetes, like the HBpresAg protein in human blood, was active in bonding with the polymerized human blood albumin receptors, but it also demonstrated the $preS_2$ region to be the recognized location of the polymerized human blood albumin receptors, i.e., the 55 amino acid sites at the N end of the HBpresAg.

Table 2. Human Polymerized Albumin Receptor Activity of HBsAg Derived From Yeast

Yeast strains	Total protein in supernatant (mg/ml)	Concentration of HBsAg or HBpresAg in supernatant (r/ml)	pHSA binding (OD 493nm)	pHSA receptor activity
DBY745-21 (carring pYGH₃21-SII)	20	2.0	2.55	++
DBY745-16 (carring pYGH₃16-S)	20	6.0	0.00	--
DBY745(control)	20	0.0	0.00	--

Following partial purification of the HBpresAg protein produced by the yeast, separate dosages of 0.25 micrograms and 0.5 micrograms were injected into Balb/c mice, and 3 weeks later more than 102 RIA units of HBsAg antibodies (Abbott kit) were found. We intend to carry out further study of its immunization ability.

Discussion

This article has reported how yeast GAP promoters stimulated the expression of surface antigen genes containing preS$_2$. The product of the expression was granular, similar to the surface antigen proteins produced by human blood surface antigens and surface antigen proteins produced by yeast. The CsCl density of the HBpresAg and HBsAg protein produced by the yeast was similar, and held specific immunogenicity as well as produced HBsAg specific antibodies that could effectively bind with polymerized human blood serum albumin. This demonstrated that the granules in the expressed product did indeed have preS$_2$ components; however, the ratio of the preS$_2$ to S in the granules will have to be analyzed further.

We constructed both HBV HBsAg[3] under yeast GAP promoter control, and an HBpresAG expression plasmid. Using identical construction methods and culturing conductions, the HBpresAg gene's level of expression was approximately 300 μg/1, which was lower than the HBsAg expression, possibly for the reason that the preS$_2$ products may have been somewhat toxic to microbe growth. Another possibility is that a certain amount of HBsAg is needed to begin with in the assembly of HBV surface antigen granules, and that only the HBpresAg was unable to form such granules. The Abbott AUSRIA kit is only able to test for surface antigen granules; it is not able to test for surface antigen polypeptides. Consequently, only the granules in the surface antigen protein derived from the yeast could be detected.

The HBpresAg gene was transformed to express in mammal cells where the HBpresAg protein and the HBsAg protein that was produced were two components that were mixed with their corresponding glucosided components. It is also possible that in mammal cells a proto-preS$_2$ product is post processed

by protease to produce a mature surface antigen HBsAg protein. Whether in yeast cells, gene transcription and its protein products carry $preS_2$ surface antigens, or whether the two are mixed components is a rather interesting question that we are currently studying.

Machida[9] et al demonstrated that the antigen precursor $preS_2$ region is able to bond with polymerized human blood serum albumin, from which they extrapolated that it is this mechanism that offers a route for the HBV virus to connect with liver cells, thereby promoting HBV virus infection of the liver. They also suggested that an HBV vaccine should carry a $preS_2$ component, not only to give it strong immunogenicity, but also so that it could interfere with the bonding of the HBV virus to liver cells as the first step in halting HBV virus infection of the liver cells. The results of our experiment also demonstrated that the surface antigen protein derived from a recombinant clone strain of yeast HBpresAg was likewise able to bond with polymerized human blood serum albumin. Therefore, the production of an HBpresAg vaccine from yeast is a system holding very good prospects.

Whether HBpresAg produced antibodies are stronger than HBsAg antibodies, and whether HBpresAg vaccine is better able to block HBV virus infection are questions awaiting thorough study.

Comrade Lu Xuanyong [7120 6693 3057] of the cellular immunity laboratory of the Zhongxin Hospital, Jing'an District, Shanghai assisted in the assay of polymerized human blood serum albumin receptor activity, for which appreciation is hereby expressed.

REFERENCES

1. Stevens, C.E., et al. Hepatitis B Vaccine. An Overview. Viral Hepatitis and Liver Disease, 1984, 275, Vyas, G.N., et al. (eds). Orlando, Fla., Grune Stratton.

2. Valenzuela, P., et al. Synthesis and Assembly of Hepatitis B Virus Surface Antigen Particles in Yeast. Nature, 1982, 298, 347.

3. Shen Luping et al. Hepatitis B Surface Antigen Expression Under Control of a Yeast GAP Promoter. To be published in SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA].

4. Neurath, A.R., et al. Hepatitis B Virus Contains Pre-S Gene Encoded Domains. Nature, 1985, 315, 154.

5. Tiollais, P., et al. The Hepatitis B. Virus. Nature, 1985, 317, 489.

6. Rall, L.B., et al. Transcription of Hepatitis B Virus by RNA Polymerase II. Mol. Cell. Biol, 1983, 3, 1766.

7. Neurath, A.R., et al. Location and Chemical Synthesis of a Pre-S Gene Coded Immunodominant Epitope of Hepatitis B Virus. Science. 1985, 224, 394.

8. Milich, D.R., et al. Enhanced Immunogenicity of Pre-S Region of Hepatitis B Surface Antigen. Science. 1985, 228, 1195.

9. Machida, A., et al. A Polypeptide Containing 55 Amino Acid Residue Coded by the Pre-S Region of Hepatitis B Virus DNA Bears the Receptor For Polymerized Human As Well As Chimpanzee Albumins. Gastroenterology, 1984, 86, 916.

10. Stibbe, W., Gerlich, W.H. Structural Relationships Between the Minor and Major Proteins of Hepatitis B Surface Antigen. J. Virol., 1983, 46, 626.

11. Ito, H., et al. Transformation of Intact Yeast Cells Treated With Alkali Cations. J. Bacteriology, 1983, 153, 163.

12. Shen Luping et al. Cu^{++} Induces Expression of Genes of External Origin Stimulated By Yeast Cu-MT Promoters. To be published in SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA]

13. Maniatis, T., et al. Molecular Cloning, Cold Spring Harbor Laboratory, 1982, 104.

14. Chen Huiying, et al. Study of the Methodology For Enzyme Immunoassay of HBsAg Polymerized Human Blood Serum Albumin Receptors, SHANGHAI YIXUE [SHANGHAI MEDICINE], 1986, 10, 596.

15. Feng Zongming [7458 1350 6900] et al. Expression of $preS_2$ Containing HBV Surface Antigen Genes. To be published in SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA].

16. Gan Renbao [3927 0086 1405] et al. Total Sequencing of Cloned adr Sub-type HBV (pADR-1) DNA. ZHONGGUO KEXUE (B Ji) [CHINESE SCIENCE (Vol B), 1986, (1), 55.

17. Michel, M.L., et al. Expression of Amplified Hepatitis B Virus Surface Antigen Genes in Chinese Hamster Ovary Cells. Biotechnology, 1985, 3, 561.

Elements Affecting Snake Venom Toxicity Studies

[Article by Guo Ningning [6753 1337 1337] and Du Yucang [2629 7187 5547], Shanghai Institute of Biochemistry, Academy of Sciences: "Localized Oxidation of Snake Venom Cytotoxin Met, and Differentiation of Biological Activity*"]

[Text] Snake venom cytotoxin can cause the depolarization of excitable cells causing smooth muscle spasms, bringing about the death of animals from heart fibrillation[1] (toxification, for short). It can also seriously damage free cells, particularly malignant cells. Yoshida's sarcoma ascitic cells are very sensitive to micrograms of the toxin; however, red cells require milligrams.[2] Whether or not efforts to use chemical modifications to produce low toxicity toxins that will retain their cytocidal activity against malignant cells seems to depend on whether the way in which the toxin expresses these two actions, and molecular action sites, are the same. The authors have reported the functional site of the toxins against mouse liver mitochondria as being in the latter's area having a high affinity for Ca^{++} bonding.[3,4] Recently, it has been further observed that cytotoxin inhibits the role of adenosine triphosphatase (ATP) in the assimilation [sic] of calcium by dog heart muscle serous membranes. One might infer that the cytotoxin's lethal affect results from an overly high concentration of calcium in the heart muscle creating (or expressed in) myofibrosis. However, experiments on Yoshida's sarcoma cells showed no calcium-ATP inhibitory role whatsoever. On the contrary, the presence of calcium interfered greatly with the cytocide malignancies.[9] Since it has been observed that the cytocide mechanism may be different than the lethal toxicology, this supports the possibility that two functional sites exist on the toxin molecules. This article reports preliminary results showing that oxidization of the residual sites was able to greatly decrease the toxicity of molecules while they retained their lysitic ability against malignancies.

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*Most of the results reported in this article were read at the 1986 meeting of the Biochemical Society

Method

1. Oxidation Modification of Cytotoxin

For information about the isolation and purification of cytotoxin C (MT-C) from the Chinese cobra (Naja Naja atra), please see the previous report.[5] Between 5 and 10 mg of MT-C was dissolved in a buffering solution consisting of either 0.1 mol/L of Tris-HCl (pH 7~9), or 0.1 mol of sodium acetate (pH 3~6) to which was added a certain amount of oxidant at a molar ratio as follows: N-chlorosuccinimide, chloramine-T, sodium hypochlorite, or hydrogen peroxide at 22 degrees C to react for 30 minutes. Following gel column chromatography (Sephadex G-25, 0.9 x 110 cm) to desalinate, the ultraviolet absorption peak was taken, and the cytotoxin was freeze dried to await analysis.

2. Bioactivity Analysis

The semi-lethal dose for mice (natural temperature MT-C at a rate of 3 μg/g of body weight) was used as the standard for lethal toxicity. Cytocidal activity was half of the time required for the toxin dose (T_{50}) to damage the aishi [5337 3044] ascitic cells in mice. Please see reference 2.

3. Oxidized Met Quantity and Location

Quantity: After oxidizing the MT-C, the method for assaying the amino acid content may be used to assay the degree of molecular oxidation, i.e., performance of a quantitative analysis of the methionine sulfoxide (MetO). However, MetO tends to desulfoxidize during acid hydrolysis; consequently, its content could not be determined with accuracy.[8] The results reported in this article were obtained by first cracking the oxidized specimen with CNBr, and then using 6 mol/L of hydrochloric acid to hydrolize it when excessive dithiothreitol (DTT) was present. Next, an amino acid component analyzer was used to determine the reversed methionine, and this amount was used to represent the MetO, i.e., the equivalent amount of molecules oxidized.

Location: MT-C has two Met residues (NH$_2$...Met^{24}.Phe^{25}. Met^{26}. Val^{27} ...COOH), and only the unoxidized Met can be cut off by the CNBr and reveal new N-terminals. If only a Phe^{25} terminal appears, this shows oxidization of the Met^{27}; if only a Val^{27} terminal appears, this shows oxidization of the Met^{24}. In order to avoid disturbing the N-terminal Leu, the MT-C's free amino-group was completely acetylated first, then the specimen was cracked by CNBr, and the DABITC method was used to assay the N-terminal.[7] Natural MT-C can only see the Val^{27} terminal; it cannot see the Phe^{25} terminal, because derivatives of the two peptides DABTH-Phe^{25}.Hse^{26} are lost with the water. However, in this assay, the MetO was zero. Therefore, only when a certain amount of oxidized methionine is found at the same time does the Val^{27} represent oxidization of Met^{24}.

Results and Discussion

1. Oxidation Modification and Cytotoxin Biological Activity

The Met residue at sites 24 and 26 on the snake venom cytotoxin molecule are
racially conservative. Figure 1a shows that as the amount of specifically
modified Met's N-chlorosuccinimide (NCS) increases, both the toxicity and
the cytocidal action against cells of the MT-C declines. This demonstrates
that these two residues may be similar to the Lys and the Trp residues at
the corresponding site in the homologous nerve toxin molecule, both being
located at the action area at which the target molecules function. However,
after oxidation with a low dosage chloramine-Ts(CAT), the cytocidal
activity of the MT-C declined clearly much more slowly than lethal toxicity
(Figure 1b), demonstrating that the two kinds of bioactivity may individually
embody different essential groups, and these groups have different degrees
of sensitivity to the oxidant.
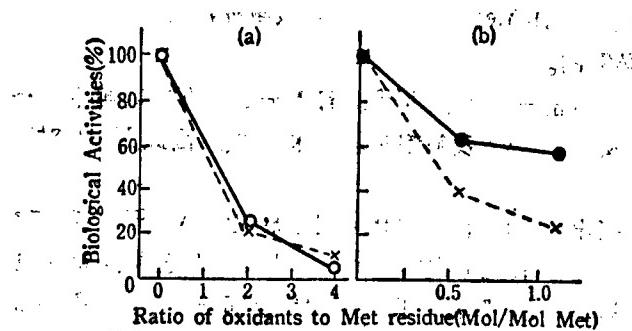
Figure 1. Effect of Oxidation on MT-C Biological Activity

1a. Percentage of cytolysis (-0-) and lethalness (-x-) of MT-C oxidized
by N-chlorosuccinimide in 0.1 mol/L Tris.Hcl buffer, pH 8.5.

1b. Percentage of cytolysis (-●-) and lethalness (-+-) of MT-C oxidized
by chloramine-T in the same buffer.

2. Oxidation Conditions

The difference in the percentage of decline of the aforestated two
bioactions were used as a criterion for comparing oxidation results achieved
by several different oxidants and at different pHs. It was noted that
NaClO, H_2O_2, and the long existing CAT served to differentiate the two
different kinds of bioaction. After processing using hydrogen peroxide
and aged CAT, the MT-C's semi-lethal dosage rose from 3 to 7.5 micrograms
per gram of mouse weight, i.e., toxicity dropped to 40 percent that of the
control. However, the specimen's cytocidal strength did not change, but
remained the same as the control. Within a pH range of 4 to 9, it was
also observed that acidic oxidation conditions were more favorable for the

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differentiation of activity. It is known that acidity helps the CAT break down to produce NaClO; therefore, the aged CAT may play an oxidation role in breaking down NaClO or H_2O_2.

3. Extent of Oxidation and Sites

The amino acid component of the oxidized products, results of the N-terminal assay, and the two kinds of biological activity are shown in Table 1. Under the oxidization conditions we employed, no oxidant was able to damage the Tyr residue from the MT-C. Aged CAT and NaClO could oxidize an average of half a Met per MT-C molecule. A CNBr cracking assay of molecules at this time showed the terminal was Val[27], demonstrating that most of the residue that was oxidized was either Met[24] or Met[25] (See the methods portion for the principles involved). It was just such modification that led to the differentiation of activity. This demonstrated that Met[24] was necessary for lethal toxicity; however, it was not necessary for cytocidal activity. When fresh CAT was used as an oxidant, the amount of MetO produced was more than one; however, both Phe[25] and Val[27] terminals appeared. This demonstrated that it was possible to modify both Met[24] and Met[26] under these conditions, leading to a simultaneous decline in both lethal activity and cytocidal activity, and demonstrating that Met[26] is necessary for cytolysis.

Table 1. Analysis of Modified Site in Oxidized MT-C

sample		bioactivity(%)		amino acid analysis		N-terminal	
oxidant	mol/mol	cytolysis	lethality	Tyr/mol	MetO/mol	Phe	Val
controll (MT-C)	—	100	100	2.8	0	—	+++
CAT	1.1/1	64	4/*1	2.8	1.1	++	++
	2.2/1	58	38*2	2.7	1.4	+++	++
aged CAT	20/1	100	45*2	2.7	0.56	±	+++
	32/1	100	46*2	2.8	0.60	—	+++
	40/1	100	50			±	+++
NaClO	4/1	100	50	2.8	0.42	+	+++
	4/1*3	100	50	2.6	0.47	+	+++
	10/1	80		2.9	0.82	+	+++

*1. Conditions of the experiment were the same as in Figure 1, but the ratio of reactants was mol oxidant to mol MT-C; the pHs of the reaction system were pH 8.5 for CAT, pH 6 for aged CAT, and pH 7.5 for NaClO.

*2. Average from three experiments.

*3. Additional fourfold aged CAT was added.

As far as the molecular structure is concerned, currently it is generally believed[10] that a hydrophobic core zone exists within the cytotoxin molecule, and except for the Met^{26}, which is located around its porifera, the Met^{26} and the three Tyr residues are all buried deeply within the hydrophobic nucleus. Consequently, it is not strange that Met^{24} is fairly sensitive to various oxidants.

Toennies pointed out some time ago that MetO could change into Met[11] within animal bodies. We had to observe for more than 2 hours when assaying toxicity, and possibly some of the MetO produced by oxidation in the molecule was reduced to Met; therefore, the semi-lethal dose of oxidized toxin may have declined due to this change. Even if the Met^{24} were to be completely oxidized, the lowering of its lethal action would not be complete. If it were possible to get around the problem of MetO reduction within the body, the extent of decline in the molecule's toxicity might be greater, i.e., the differentiation of the two activities might be more complete. Efforts to complete irreversibly the independent modification of Met^{24} are underway.

REFERENCES

1. Condrea, E.: Membrane-active Polypeptides From Snake Venom. Cardiotoxins and Haemocytoxins Experientia, 1974, 30, 121.

2. Du Yucang: The Role of Chinese Cobra Cytotoxin-C in In Vitro Cytocide of Yoshida's Sarcoma Cells. SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1984, 6, 165.

3. Gu Benxian [7357 2609 6343] and Du Yucang: Study of the Effect of Chinese Cobra Cytotoxin-C on the Action Sites of Mouse Liver Mitochondrial Membranes, SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1984, 6, 165.

4. Gu Benxian and Du Yucang: Cell Cytotoxin Inhibition of Mitochondria Passages for Assimilating [sic] Calcium, SHENGWUHUAXUE ZAZHI [BIOCHEMISTRY MAGAZINE], 1985, 1, 65.

5. Du Yucang et al: Isolation and Identification of a New Snake Venom Cytotoxin D_1, SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1985, 17, 199.

6. Gross, E., and Witkop, B.: Nonenzymatic Cleavage of Peptide Bond: The Methionine Residues in Bovine Pancreatic Ribonuclease, J. Biol. Chem., 1962, 217, 1856.

7. Wu Wenyu and Du Yucang: Amino Acid Sequence of Cytotoxin D_1, SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHIMICA et BIOPHYSICA SINICA], 1984, 16, 310.

8. Glazer, A.N., et al: Chemical Modification of Proteins: Selected Methods and Analytical Procedures. Laboratory Techniques in Biochemistry and Molecular Biology, 1975, 27, Work, T.S., and Burdon, R.H. (eds), Presser: North-Holland Elsevier.

9. Hider, R.C., and Khader, F.: Biochemical and Pharmacological Properties of Cardiotoxins Isolated From Cobra Venom, Toxicon, 1982, 20, 175.

10. Lauterwein, J., and Wuthrich, K.: A Possible Structural Basis for the Different Modes of Action of Neurotoxins and Cardiotoxins From Snake Venoms, FEBS Letters, 1978, 93, 181.

11. Toennies, G.: The Oxidative Conversion of Casein Into Protein Free of Methionine and Tryptophene. J. Biol. Chem., 1942, 145, 667.

Detection of Colonization Factor Antigen I (CFA/I) of Enterotoxigenic
E. coli

[Article by Li Shuqin [2621 3219 3830] et al., Institute of Biotechnological Sciences, Academy of Military Medical Sciences, Beijing: "Enzyme-linked Immunosorbent Assays for Detection of CFA/I of Enterotoxigenic Escherichia coli"]

[Text] Abstract: This paper describes the procedure for preparing anti-CFA/I serum from ETEC strain E-100 and the results of the detection of CFA/I of clinically isolated ETEC strains using ELISA. This method is rapid, sensitive, specific and convenient. It not only can detect CFA/I-carrying wild types, but also recombinant clones producing CFA-I subunit. Furthermore, the serum thus prepared cross reacts with other enteric diarrheal pathogens. In general, it might provide an efficient approach to both epidemiological investigation and clone selection.

Key words: ETEC, CFA/I, Antiserum, ELISA

Enterotoxigenic E. coli (ETEC) is one of the important pathogens that induce diarrhea in babies as well as in travellers. The bacteria first adhere to the epithelial cells of the small intestine via the colonization factor antigen (CFA) on the bacterial surface, colonize, and proliferate to cause the disease. CFA is therefore a pre-requisite condition for the virulence of ETEC. Recently, CFA is drawing attention from many domestic as well as overseas investigators. In order to study the biological characteristics of CFA, numerous detection methods have now been established including blood coagulation method, ammonium sulfate aggregation method, electron microscopy, immuno-electron microscopy, and indirect immuno-fluorescent technique. The first two show less specificity and furthermore, are affected by many factors. The latter three are complicated and tedious in manipulation, making them inconvenient for a large scale investigation. During gene manipulation, in order to select a gene recombinant clone, there should be a simple and rapid technique suitable for mass detection. We have established an enzyme-linked immunosorbent assay (ELISA) technique, with which 157 clinical ETEC isolates were tested, and found that 13 strains

or 8.3 percent were CFA/I positive. In the laboratory, we used the method for the screening of recombinant transformants for CFA/I gene subunit, and obtained good results.

Materials and Methods

1. Bacterial Strains. E. coliH10407 is a CFA/I producing standard strain, E. coliE-100 is a domestically isolated CFA/I producing strain, and E. coliE-100P is a derivative of strain E-100. Clinically isolated 157 ETEC strains and other enteric diarrheal pathogens were also used.

2. Materials. Buffer solution: 0.05mol Na_2CO_3-$NaCO_3$, pH 9.6; and antigen dilution medium and washing solution: PBS-Tween 20, pH 7.4 (NaCl 0.8gm, KCl 0.02gm, $NaHPO_4 \cdot 12H_2O$ 0.29gm, KH_2PO_4 0.02 gm, Tween 20 10µl, add water to 100ml) were prepared and stored at 4°C. Enzyme conjugated antibody dilution solution was prepared just before use by adding bovine serum albumin to 1 percent to PBS-Tween 20, pH 7.4. Base solution: 0.1mol phosphate buffer, pH 6.0 was stored at 4°C, and just prior to use, 2ml were taken to dissolve 4mg of diaminobenzene, and added with 16 ul of 30 percent H_2O_2 in the dark.

3. Method. (1) Two methods were used for the preparation of bacterial suspension to be tested. One was to disrupt bacteria by ultrasonic sound: after overnight growth on a CFA agar plate, the bacteria were suspended in PBS to a concentration of 10^{10} bacteria/m., disrupted with ultrasonic sound waves for 7 minutes, and centrifuged for 20 minutes at 18,000 rpm to remove bacterial debris, and the supernatant was diluted two fold for the test. The other was prepared by a heating method: after overnight growth on a CFA agar slant or spot-growth on an agar plate (20 colonies can be grown on a plate 100mm in diameter), the bacteria from the former can be suspended in 1 ml physiological saline solution and the latter in 0.1 ml physiological saline solution for each colony, heated at 60°C for 30 minutes in a water bath, and diluted two fold just before use.
(2) Preparation and purification of antiserum were done by the method of Evans, et al.[1] with modification. Rabbits were immunized six times intravenously with formalin-inactivated E. coliE-100, with an injection every 3 days. The dose for the first injection was 10^6 formalin-treated bacteria (0.1 ml), 2×10^6 (0.2 ml) for the second and the third injection, and the dose for the last three injections was 10^9 (1 ml)/injection. Bleeding was done 2 weeks after the 6th injection. The antiserum obtained was repeatedly absorbed with E. coliE-100P. The antiserum was first boiled for one hour to kill bacteria, and washed three times with PBS. In 10 ml of antiserum, 5 g of wet bacteria were added, mixed well, incubated at 37°C for 4 hours, and then at 4°C in a refrigerator overnight. After centrifugation, the supernatent was readsorbed with the bacteria, and the procedure was repeated two-three times until no aggregation of E. coliE-100 in the antiserum was shown. Next, the absorbed antiserum is again absorbed three times using the above method with live E. coliE-100P grown in brain-heart infusion medium. The antiserum was then further purified by mixing with DEAE-Sephadex A50 and filtration[2] for the use in ELISA test.

(3) ELISA detection of CFA/I was carried out using the CFA/II detection method of Mullany, et al.[3] The enzyme-conjugated antibody was prepared according to the $NaIO_4$ method[4]. Based on the comparison of the value (P) of samples obtained and the value (N) of negative control obtained at 492 nm wavelength, it was judged to be positive when P/N>5.

Results and Discussion

1. Determination of Titer and Purity of the Antiserum

Slide agglutination test was used to determine the titer of the antiserum obtained by this immunization protocol. The result showed that the agglutination titer of the antiserum obtained was 1:8000 against CFA/I producing E. coliH10407 and E-100, and it was also 1:8000 against CFA/I non-producing strain E-100P; whereas the titer of the antiserum tested against K88 pili carrying ETEC derived from pig was 1:32, while against CFA/II containing ETEC derived from human was 1:16. The titer of the antiserum tested against often used laboratory strain of E. coliK-12 was 1:4. However, after the absorption with E-100P, the antibodies reacted against the H and O antigen and other common antigens were removed. The antiserum after absorption no longer agglutinated against CFA/I non-producing E. coli, yet it still agglutinated CFA/I producing E. coli with a relatively high titer (1:256).

2. The Effect of Bacterial Suspensions Prepared by Various Methods on the Test Results

The results of the test on the effect of antigens prepared by various methods indicated that under a condition of similar bacterial concentration, similar values ($A_{492nm} \simeq 1.50$) were obtained whether or not the antigen was prepared by ultrasonic disruption or heat treatment when the standard positive strain was used. However, the P/N value obtained with the ultrasonic disrupted antigen was slightly higher than that obtained with the heat treated antigen, suggesting the quality of the sample obtained with the ultrasonic disruption was slightly better. On the other hand, the ultrasonic disruption procedure is relatively more complex, and is therefore unsuitable for a large scale selection and large scale survey of the sample. In contrast, the heat treatment procedure for the preparation of bacterial suspension is simple and easy to use. Especially, the sample to be tested can be spot-grown on agar plates: on a plate medium 90 mm in diameter, 10-20 colonies can be inoculated and grown. This method saves time, and is economical and suitable for a large scale test. In practical application, therefore, we recommend the use of the heat treatment method to deal with samples.

3. Test with Other Diarrheal Pathogens

Using the present method, we also carried out tests on other diarrheal pathogens such as Shigella, invasive E. coli, V. cholera, Salmonella typhimurium, and ETEC containing other pilus types, etc. The results showed no cross reaction to these bacteria, indicating that the present method exhibits very good specificity and reliability.

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4. Application of ELISA to the Assay of Clinical Isolates and for the Selection of Recombinant Clones

In order to search for and prepare a gene probe for CFA/I, we have isolated and purified plasmid DNA containing CFA/I gene from E. coliH10407, and after digestion with restriction endonuclease BglII, recovered a 3.2 md DNA fragment, which was recombined with BamHI digested vector pUC18. After the initial screening on a selective medium, the recombinant clones were further screened using the ELISA introduced here. The result obtained was quite good. Although the recombinant obtained by the above method contains only the coding region for CFA/I functional unit protein, i.e., it can only express a subunit of CFA/I protein, which cannot form mature pili, the ELISA method established here can detect the necessary recombinant clones. Also, using the method, we tested 157 E. coli strains isolated from diarrheal patients' feces, and found that 13 strains were positive, which means that 8.3 percent of the samples tested were CFA/I containing strains.

The distribution of ETEC is very wide and its virulence high. However, China still lacks a rapid and specific detection method for effective detection of bacteria containing CFA/I. This article introduced a clinical ETEC assay technique and offers a new approach for epidemiological studies. It can be an effective method for the screening of CFA/I gene clone in gene recombination work.

References

1. Evans, D.G., et al., Plasmid-controlled colonization factor associated with virulence in Escherichia coli enterotoxigenic for humans. Infect Immun. 1975; 12:656.

2. Yang Jiping, et al. A simple and rapid method for preparation of serum r-globulin protein. Clinical Immunology and Experimental Immunology 1980; 1(3):18.

3. Mullany, P., et al., Expression of plasmids coding for colonization factor antigen II (CFA/II) and enterotoxin production in Escherichia coli. J Gene Microbiol. 1983; 129:3591.

4. Yu He, et al., Clinical Immunology Technique. 1st ed., Shanghai Science Technology Publication Co., Shanghai, 1982; pp 206-207.

Study on Synthesis of Morphine Analogues

[Article by Zhang Yongmin [1728 0516 3046], Zhang Lihe [1728 4409 0735] and Liu Weiqin [0491 4850 0530] of the School of Pharmacy, Beijing Medical College]

[Text] Morphine (I) has a very strong analgesic function; however, its highly addictive nature greatly restricts its utility. Since confirmation of opium receptors and discovery of autogenous opium peptides in the seventies, opium receptor research has made much progress. This has energetically advanced research into morphine analogue compound substitutes.

Concerning alteration of the morphine structures, a great deal of research has been done. Basically this involves simplifying the molecular structure of morphine. Breaking of the ring is a very common method[1]. It is apparent from what is reported in the literature that there is very little research into hydrodibenzofuran-type structures minus the C and D rings. The coupling of salicylic acid metabolism and oxidation produces the dibenzofuran structure. This interested us in the study of hydrobenzofuran-type compounds. Hosokawa and others have reported using the oxidation method of ring construction to obtain a variation of hydrobenzofuran compounds (II)(III)(IV)[2]. In order to obtain structures even more similar to morphine molecules, we designed a hexahydrodibenzofuran substitute, i.e., 4,6-dihydroxy-9bβ as a subject of research. A.G. Schultz and others have reported the method of using coronization reactions in the preparation of this type of compound[3]. We then developed a highly stereochemically selective, new synthesized 2-oxy-3aα-(2',3'-dimethoxymethylenoxyphenyl)-7α-iodo octahydrobenzofuran (V), its chemical structure has been determined by IR, NMR, MS, elemental analysis, etc.[4]

Under conditions of basicity, when (V) undergoes methanolysis the epoxide (VI) is formed. The latter (VI) is very active, inner-molecular ring formation reactions occur easily, forming hexahydrodibenzofuran ester (VII). Upon hydrolysis of (VII), a certain amount of acid (VIII) is produced. The ^{1}HNMR spectrum of acid (VIII) has a double peak of 4.6 ppm. This is the absorption of the 4a proton. Based on the coupling constant J = 7 Hz, the 4a proton and the position 4 proton should change to antiform position. To confirm

this judgment, we hydrolyzed the amide bond of compound (IX). This also yielded hexahydrodibenzofuran (X). The stereochemical structure of compound (IX) has already been determined by X-ray diffraction, therefore, the stereochemical structure of acid (X) is already known[5]. After careful comparison of all kinds of spectrograms of acid (VIII) and acid (X), it was discovered that they are entirely the same. Therefore, we believe that they are the same compound.

Because compound (VII) has an ester base side chain, it changes easily. This facilitates preparation of hexahydrodibenzofuran derivatives with different side chain structures. From this a series of new compounds can be obtained, not only in order to establish a foundation for research into the relationship between structure and effect, but possibly also to select our compounds with even better bioactivity.
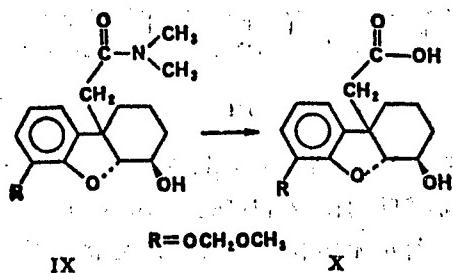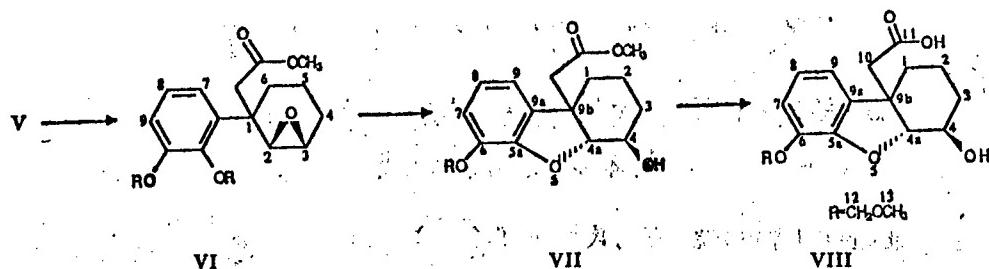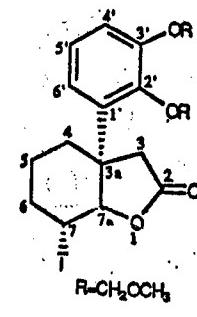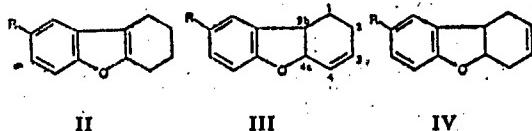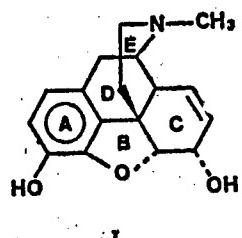
From this we can obtain the following reaction mechanisms:
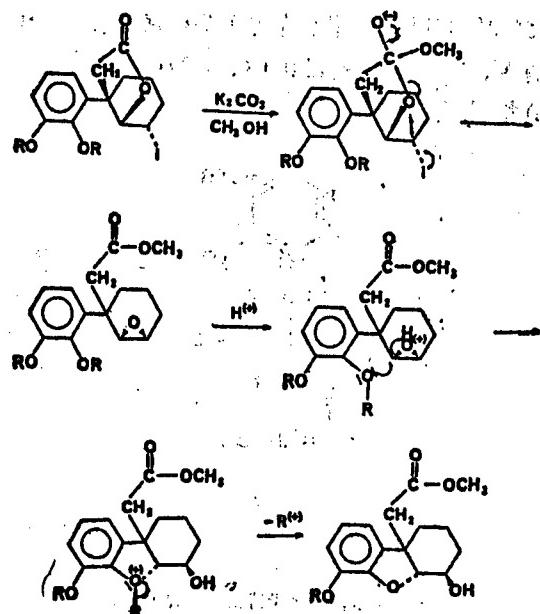
Experimental Procedure Section

^{1}HNMR spectrum was determined by "VARIAN"T-60 or "BRUKER"WP200sY nuclear
magnetic resonance instrument, TMS was the internal standard. ^{13}CNMR spectrum
was determined by "VARIAN"CFT20 nuclear magnetic resonance instrument, $CDCl_3$
was the internal standard. Mass spectra were determined by GC/MS "NERMAG"
R 10-10 mass spectrograph. The elemental analysis instrument was a "PERKIN
ELMER"-240 model, infrared spectra were determined by a "PERKIN ELMER"-177
model spectrograph, melting points were determined by a "METTLER"FP51
instrument.

1) Preparation of 2-[1-(2',3'-dimethoxymethylenoxy)-2,3-β-epoxy-1-cyclohexyl]-
methylacetate (VI).

Use 462mg(1 mmol) octahydrobenzofuran (V), dissolve in 20ml methanol, add
138mg(1 mmol) potassium carbonate, place reactant in dark at room temperature
and stir for 12 hours, pour in 100ml water, use ethyl acetate to extract
(3x50ml), after washing the organic elements with water (50ml), use
dehydrated sodium sulfate to dry. Reduce pressure to evaporate the solvent.
Use silica gel to separate the column layers, use dichloromethane--ethyl
acetate to elute, obtaining 256mg(VI) (oil-like material) with a 70 percent
recovery rate.

^{1}HNMR (CD_3Cl) δppm (200MHz): 1.1∿2.1 (m, 6H, 3 CH_2); 3.02 (d, AB, J = 16Hz,
1H, -$\overset{O}{\overset{\|}{CH_2C}}$O-; 3.30 (d, AB, J = 16Hz, 1H, -$\overset{O}{\overset{\|}{CH_2C}}$O-; 3.40 (m, 1H, H_3); 3.48 (S, 3H,
OCH_3); 3.51 (S, 3H, OCH_3); 3.60 (S, 3H, OCH_3); 3.78 (d, J = 4Hz, 1H, H_2); 5.17
(S, 2H, OCH_2O); 5.20 (2d, AB, 2H, OCH_2O); 7.00∿7.15 (m, 3H, H_7, H_8, H_9).

Elemental analysis $C_{19}H_{22}O_7$(366.42), calculated value% C 62.23, H 7.15; determined value% C 62.27, H 7.01%.

2) Preparation of 4β-hydroxy-6-methoxymethylenoxy-9bβ-methoxycarbonylmethyl hexahydrodibenzofuran (VII).

Dissolve 366mg(1 mmol) 2-[1-(2',3'-dimethoxymethylenoxy-2,3-β-epoxy-1-cyclohexyl]-methylacetate (VI) in 20ml methanol, add a catalytic amount of acetic acid, heat and reflux the reactant for 10 hours, TLC indicates the reaction is complete. Evaporate the solvent under reduced pressure, use silica gel to separate column layers, elute with ethyl acetate, obtaining 290mg(VII) (oil-like material) with a 90 percent recovery rate.

HNMR (CDCl$_3$) δppm (200MHz): 1.1∿2.1 (m, 6H, 3CH$_2$); 2.60 (2d, AB, J = 15Hz,

2H, CH$_2\overset{\overset{\text{O}}{\|}}{\text{C}}$O-); 2.65 (m, 1H, exchange with D$_2$O OH); 3.50 (S, 3H, OCH$_3$); 3.55 (dd, J$_1$ = 7Hz, J$_2$ = 10Hz, H$_4$); 3.60 (S, 3H, OCH$_3$); 4.60 (d, J = 7Hz, H$_{4a}$); 5.27 (2d, AB, 2H, OCH$_2$O); 6.70∿7.00 (m, 3H, H$_7$, H$_8$, H$_9$). Elemental analysis $C_{17}H_{22}O_6$(322.36), calculated value% C 63.34, H 6.88; determined value% C 63.68, H 7.11.

3) Preparation of 4β-hydroxy-6-methoxymethylenoxy-9bβ-carbonylmethyl-hexahydrobenzofuran (VIII).

Dissolve 322mg(1 mmol) methylhexahydrobenzofuran (VII) in 10ml ethanol, add 10ml of 20 percent sodium hydroxide aqueous solution, heat and reflux the reactant for 6 hours, evaporate the ethanol under reduced pressure, use 6mol/L hydrochloric acid to neutralize the remaining solution to pH6, use ethyl acetate to extract (3x20ml), combine organic elements, wash with water, use dehydrated magnesium sulfate to dry. After evaporation of the solvent under reduced pressure, 320mg of solid material remains, after recrystallization (ethyl acetate--petroleum ether) 317mg of white crystallized (VIII) is obtained, a recovery rate of 97 percent. mp 75.3°C, elemental analysis indicates this compound contains one molecule of crystallized water: $C_{16}H_{20}O_6\cdot H_2O$ (326.35), calculated value% C 58.89, H 6.79; determined value% C 59.00, H 6.64. ^{1}HNMR (CDCl$_3$) δppm (60MHz): 1.1∿2.1

(m, 6H, 3 CH$_2$); 2.60 (S, 2H, CH$_2\overset{\overset{\text{O}}{\|}}{\text{C}}$-O-); 3.50 (S, 3H, OCH$_3$); 3.65 (m, 1H, H$_4$); 4.70 (d, 1H, J = 7Hz, H$_{4a}$); 5.25 (2d, 2H, J = 7Hz, OCH$_2$O); 6.30 (S, 2H, exchange with D$_2$O, 2xOH); 6.70∿7.10 (m, 3H, H$_7$ H$_8$ H$_9$). IRν$_{max}^{KBr}$Cm^{-1}3430(OH), 1713(CO); 777,735(1,2,3-these three replace ben). MS (E·I)m/z 308 (M$^+$), 290,231,45. ^{13}CNMR (CDCl$_3$) δppm (20 MHz) 19.4(t, C$_2$); 29.5 and 29.7(2t, C$_1$ and C$_3$); 44.1(t, C$_{10}$); 47.8(s, C$_{9b}$); 56.0(q, C$_{13}$); 71.5(d, C$_4$); 92.4(d, C$_{4a}$); 95.2(t, C$_{12}$); 116.2(d, O$_7$); 117.0(d, C$_9$); 121.7(d, C$_8$); 134.8(s, C$_{9a}$); 142.3(s, C$_{5a}$); 147.1(s, C$_6$); 174.8(s, C$_{11}$).

4) The hydrolysis reaction of compound (IX).

Dissolve 335mg(1 mmol) 4β-hydroxy-6-methoxymethylenoxy-9bβ-N, N-dimethyl-aminecarbonylmethylhexahydrodibenzofuran (IX)[5] in 10ml ethanol, add 10ml of

20 percent sodium hydroxide aqueous solution, heat and reflux the reactant for 24 hours. Evaporate the ethanol under reduced pressure. Use ethyl acetate to extract (2x10ml) of the remaining aqueous solution. After separating out the water, use 6mol/L hydrochloric acid to neutralize to pH5, again use ethyl acetate to extract (3x20ml), combine organic elements, use dehydrated magnesium sulfate to dry. After evaporation of solvent under reduced pressure, 305mg of solid material is obtained. Use ethyl acetate-- petroleum ether to recrystallize, 294mg of white crystal is obtained, for a recovery rate of 90 percent. Elemental analysis indicates that this compound contains one molecule of crystallized water: $C_{16}H_{20}O_6 \cdot H_2O$ (326.35), calculated value% C 58.89, H 6.79, determined value% C 58.97, H 6.57, thus determined IR, ^{1}HNMR, ^{13}CNMR and MS spectra are the same as compound (VIII).

References

1. Casy, A.F., et al., Opioid Analgesics, Chemistry and Receptors. New York and London: Plenum Press, 1986:1-518.

2. Hosokawa, T., et al., Oxidative cyclisation of Z-allylphenol by palladium (III) acetate, changes in product distribution. J. Org. Chem., 1978, 43 (14):2752.

3. Schultz, A.G., et al., Total synthesis of 1-Lycoramine. J. Am. Chem. Soc., 1977, 99(24):8065.

4. Zhang Yongmin, Syntheses d'analogues morphiniques a squelettes tetrahydro ou hexahydrodibenzofuranniques, These de Doctorat es Sciences Pharmaceutiques. Universitie Paris-Sud 1986; serie E. No 253:1-188.

5. Labidalle, S., et al., Synthese d'analogues morphiniques a squelette tetrahydrodibenzofurannique. Tetrahedron Lett 1986; 27(25):2861.

Regeneration of Brevibacterium Flavum Protoplasts

[Article by Zheng Jiaan [6774 0857 1344], Zhong Guixiang [6988 2710
7449] and Yuan Mingxiong [5913 2494 3574] of the Department of
Bioengineering, Huazhong University of Science and Technology: "On the
Conditions for Formation and Regeneration of Brevibacterium Flavum
Protoplasts"; Received April 21, 1986]

[Text] [Abstract] The conditions for raising the regeneration rate of
Brevibacterium flavum protoplasts were established. Experimental
results demonstrated that the optimal regeneration rate of our procedure
was 150 percent higher than that reported by Qiao Bao Yi, et al[1] and 67
percent higher than that reported by H. Kaneko, et al[2].

Key Words: Brevibacterium Flavum, Protoplasts, Rate of Formation, Rate
of Regeneration

Brevibacterium flavum is one of the more important bacteria frequently
used in production of amino acids[3]. Because no transformation,
transduction or conjugation mechanism has ever been found in this
particular bacterium, the study of protoplast formation, regeneration
and fusion of Brevibacterium flavum is of great theoretical as well as
experimental significance. The fusion of protoplasts and genetic
recombination of Brecibacterium flavum were reported previously[2]. More
recently, a preliminary study of the formation, regeneration and fusion
of the protoplasts of Bacillus beijing, a bacterium similar to
Brevibacterium flavum, was also published[1]. However, the reported
regeneration rates were relatively low: close to 30 percent for the
former and approximately 20 percent for the latter.

A prerequisite condition for the fusion and cloning of Bacterium flavum
protoplasts is to raise their regeneration rate to an acceptable level.
Herewith we will describe our study of the optimal conditions for the
formation and regeneration of the protoplasts of glutamic acid
Brevibacterium flavum T_{6-13} and procedures to improve its regeneration
rate.

I. Materials and Methods

1. Materials

(1) Bacterial strain. T_{6-13} strain of glutamic acid Brevibacterium flavum was obtained from Wuhan City Monosodium Glutamate Factory.

(2) Media

(i) Basic Cultural Medium

$(NH_4)_2SO_4$	10g	NaCl	50mg
KH_2PO_4	1g	$MnSO_4 \ H_2O$	2mg
$MgSO_4 \ 7H_2O$	0.4g	$FeSO_4 \ 7H_2O$	2mg
Glucose	20g	Biotin	50μg
Urea	3g	Thiamine	200μg
H_2O	1000ml	pH 6.5	

(ii) General Cultural Medium

Basic cultural medium supplemented with 0.1 percent yeast extraction.

(iii) Regeneration Cultural Medium

General cultural medium supplemented with 0.25 M sucrose and 0.25 M sodium succinate. Solid and soft agar media contained 1.2 percent and 0.4 percent agar (the domestic Yellow Sea brand), respectively.

(3) Other Solutions and Fluids

(i) The Dilution Fluid

Sucrose	0.25M	K_2HPO_4	0.02M
Sodium Succinate	0.25M	KH_2PO_4	0.11M
EDTA	0.001M	$MgCl_2$	0.01M

(ii) Penicillin Solution

The stock solution contained 4,000 u/ml of penicillin G and was kept at 4°C. (Product of Northern China pharmaceutical co.)

(iii) Zymolysis Fluid

The eggwhite lysozyme was the product of the Second Fowl Eggs Factory, Shanghai Fowl Egg Products Company.

2. Methods

(1) Cultural Conditions

A loopful of the culture on a stock slant was transferred into 10ml of the basic medium and incubated at 30°C overnight on a shaker. An aliquot of 0.2ml was then inoculated into 20ml of general medium and the incubation was continued until the early exponential phase of growth.

(2) Protoplast Formation

In the early exponential phase, the cultural medium was treated with a predetermined volume of penicillin solution to give a final concentration of 0.5u/ml of penicillin G. After shaking for 2 more hours, the medium was centrifuged at 2,000 rpm for 10 minutes. The cells were harvested and resuspended in 10ml of zymolysis fluid. The suspension was then incubated at 30°C, without shaking, until the protoplast formation was detected (monitored by microscopic observation and the water-sensitivity test).

(3) Protoplast Regeneration

The supernatant of the zymolysis fluid suspension was removed by centrifugation. Dilution fluid (10ml) was then added to the digested protoplasts; the suspension was diluted 10-fold with the dilution fluid. The thinned down broth was then spread on regeneration medium agar plate and incubated at 30°C temperature. In the meantime, for the determination of the number of viable cells, the 10-fold diluted broth was further diluted with low osmotic pressure fluid (sterilized water). The twice diluted broth was spread on the ordinary medium agar plate and incubated at 30°C temperature. The number of colonies appearing on the plate was then counted.

(4) Determination of the Formation Rate and Regeneration Rate of the Protoplast

This was conducted following the procedure as described in the literature[1].

II. Results

1. The Effect of Pretreatment With Penicillin Solutions of Various Concentrations on the Growth of T_{6-13} Cells

After a 6-hour incubation, aliquots of the cell culture were treated with appropriate volumes of penicillin solution so that the final concentrations of penicillin G were 0.05, 0.1, 0.2, 0.4 and

0.6u/ml respectively. After 2 hours, the optical densities and numbers of colonies of the cultural samples were measured and compared with those of the medium which had not been spiked with the penicillin solution. The results were presented in Table 1.

Table 1. The Effect of Penicillin Pretreatment on the Cell Growth

Penicillin Concentration (u/ml)	0	0.05	0.1	0.2	0.4	0.6
OD Value	0.22	0.22	0.2	0.178	0.137	0.107
Number of Colonies per ml	1.3×10^8	1.28×10^8	1.07×10^8	7.3×10^7	5.5×10^7	3.6×10^7

As shown in Table 1, growth inhibition was observed with a penicillin concentration of 0.1 u/ml; the effect became more pronounced for samples containing a penicillin concentration of 0.2 u/ml or higher.

2. The Effect of Incubation Periods Before Penicillin- Spiking on the Formation and Regeneration of Protoplasts

Cultural samples were incubated, and treated with 0.05 u/ml penicillin G at different intervals, then incubated for another 2 hours. The observed protoplast formation and regeneration rates are plotted in Figure 1.
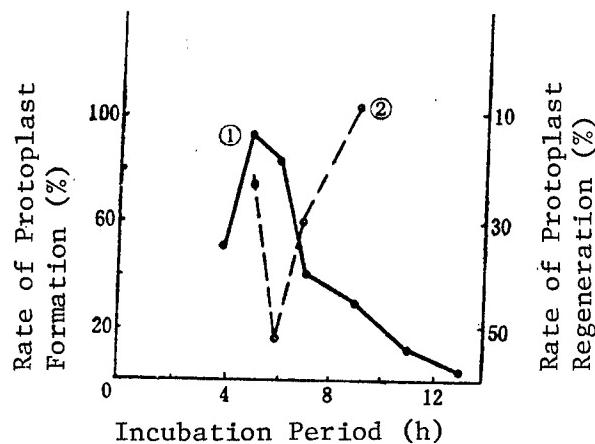
Figure 1. The Effect of Incubation Periods Before Penicillin-Pretreatment on the Formation and Regeneration of Protoplasts

Figure 1 indicates that the formation rate peaked when the broth was inoculated with penicillin G after a 5 - 6 hour incubation period (curve 1), while optimal regeneration rate was obtained when the

penicillin solution was added to the broth at 6 hours (curve 2), which corresponded to the early exponential phase of the growth curve of T_{6-13} cells.

3. The Effect of Incubation Periods of Penicillin Pretreatment on Protoplast Formation

The cultural medium was treated with penicillin G (0.3 u/ml) for 1 - 3 hours and the formation rate was measured at intervals. The result as shown in Figure 2.
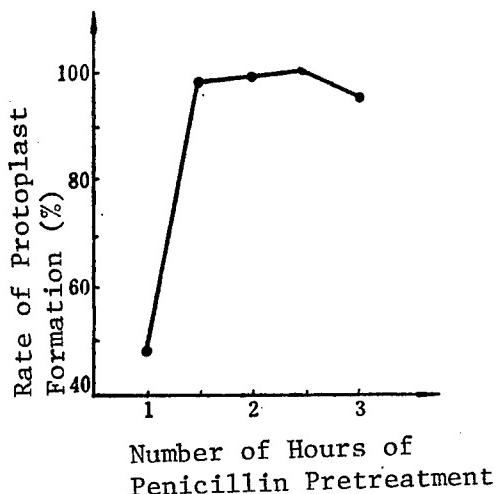

Figure 2. The Effect of Incubation Periods of Penicillin Pretreatment on Protoplast Formation

Best results were obtained, as shown in Figure 2, when the broth was treated with penicillin for 1.5 to 2.5 hours; therefore, in the present study, all the penicillin pretreatments were conducted for 2 hours.

4. The Effects of Agar Concentration and Culturing Conditions on the Regeneration Rate of Protoplasts

The effects of agar concentration and the efficiencies of overlay medium versus basal medium were studied using broth samples which had been pretreated with 0.3 u/ml of penicillin G for 2 hours, then digested with lysozyme for 15 hours. Experimental results clearly indicated that best results were obtained at an agar concentration of 1.4 percent and the basal medium is more efficient than the overlay medium.

5. The Effect of Concentration of Lysozyme on the Formation of Protoplasts

The effect of lysozyme concentration on protoplast formation was
studied on broth samples pretreated with 0.3 u/ml of penicillin G for 2
hours. The results is shown in Figure 3.
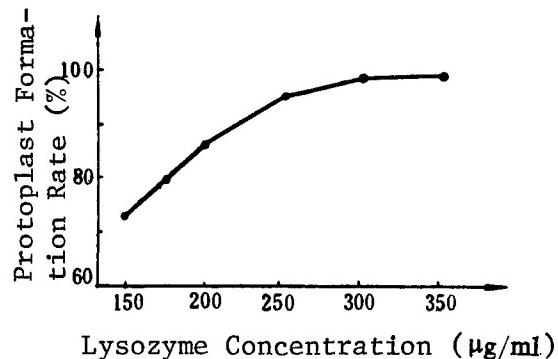
Figure 3. The Effect of Lysozyme Concentration on the Formation of
Protoplasts

As shown in Figure 3, the regeneration of protoplasts was enhanced
beginning at 150 √g/ml of lysozyme; the regeneration rate rose with
increasing lysozyme?concentration and plateaued near 100 percent at
300 √g/ml of lysozyme.

6. The Effect of the Length of Lysis Period on the Formation and
Regeneration of Protoplasts

The cultural broth which had been pretreated with 0.05 u/ml of
penicillin for 2 hours, was digested with 150 √g/ml lysozyme. The rate
of formation and regeneration were measured at intervals and tabulated in
Table 2.

Table 2. The Effect of zymolysis Time on the Formation and Regeneration
of Protoplasts

Zymolysis Time (h)	6	7	8	12	16
Rate of Formation (%)	70	80	89	94	100
Rate of Regeneration (%)	10	22	53	23	7.2
Rate of Formation x Rate of Regeneration	700	1760	4717	2162	720

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As shown in Table 2, the optimal zymolysis time is 8 hours, when the protoplast formation rate was close to 90 percent where as the regeneration rate was well above 50 percent.

7. The Effect of Glycine on the Formation and Regeneration of Protoplast

Table 3 compares the protoplast formation and regeneration rates between a cultural medium (containing 0.3 u/ml of penicillin G and 150μg/ml lysozyme) supplemented with 6.25 mg/ml glycine and another one without added glycine.

Table 3. The Effect of Glycine on the Formation and Regeneration of Protoplasts

Glycine	Formation (Rate %)	Regeneration Rate (%)	Formation Rate x Regeneration Rate (10^{-4})
No	87.3	3.1	271
Yes	95.5	13.5	1289

As shown in Table 3, glycine raised the rates of formation and regeneration of the protoplast significantly in the medium; this effect was even more pronounced in protoplast regeneration. In addition, microscopic observation revealed that the rate of protoplast released from the cell was further accelerated as well.

The Effect of Penicillin Concentration on the Formation and Regeneration of Protoplasts

Penicillin G Concentration (u/ml)	Formation Rate (%)	Regeneration Rate (%)	Formation Rate x Regeneration Rate (10^{-4})
0.05	74	48	3552
0.10	87	9.7	843.9
0.30	99.1	3.4	336.9
0.50	100	0	0

Table 4 shows that the rate of protoplast formation increased whereas the rate of protoplast regeneration decreased with increasing concentrations of penicillin G. At a penicillin G concentration of 0.5 u/ml, the formation rate rose to 100 percent, yet the regeneration

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rate fell to zero. A concentration of 0.05 u/ml for penicillin G was recommended in order to maximize the product of the protoplast formation and regeneration rate.

III. Discussion

(1) Penicillin treatment made the glutamic acid Brevibacterium flavum protoplasts susceptible to the action of lysozyme. It was recognized that the penicillin treatment of the cells in their exponential growth phase had a remarkable effect on protoplast formation[2]. Our experimental results also illustrated that exposure of the cells to penicillin G in the early exponential phase promoted the protoplast formation. Since during this phase of rapid cell growth, as a result of accelerated metabolism, the cell walls of Brevibacterium flavum became increasingly vulnerable to the zymolysis by lysozyme molecules, a condition favorable for protoplast formation and for raising the regeneration rate as well.

(2) The efficiency of the formation of the protoplast and its ability to regenerate were intensely affected by the celluar protoplasting conditions. Factors which governed the celluar protoplasting process usually exerted great effects on protoplast regeneration. Vigorous protoplasting conditions afforded high protoplast formation rates, yet low regeneration rates. Therefore, milder protoplasting conditions were generally favored for yielding satisfactory numbers of colonies and regeneration rates. The conditions established in this study induced a protoplast formation rate and a regeneration rate greater than 80 percent and 50 percent, respectively.

(3) The formation and regeneration of protoplasts were also closely affected by the physiological state of the cells. Factors, such as the age of the strain, the incubation time of the strain when treated with penicillin as well as the extent of zymolysis, played important roles in affecting the outcome of protoplast formation and regeneration.

(4) Addition of glycine in the penicillin pretreatment facilitated, not only the release of protoplasts from the cells (a possible explanation was that glycine inhibited the binding of alanine to polysaccharides), but also the formation of protoplasts; therefore its regeneration as well.

References

1. Qiao Baoyi (0829 1405 5030) et al., A preliminary Study of the Formation, Regeneration and Fusion of Bacillus Protoplasts; J. of Microbiology, Vol. 23, No. 1, pp 33-43 (1983).

2. Kaneko, H., et al., Fusion of Protoplasts and Genetic Recombination of Brevibacterium Flavum; Agric. Biol. Chem., Vol. 43, No. 5, pp 1009-1013 (1979).

3. Yamada, K., et al., The Microbial Production of Amino Acids; New York, Halsted Press (1972).

Design of Real-Time Software for Programmable Telemetry/Computer System

[Article by Guo Fushun [6753 4395 7311] and Luo Xin [5012 2500] of the System Software Teaching and Research Section, Harbin Institute of Technology; manuscript received December 1987]

[Text] Abstract

Telemetry systems have been combined with computers to make modern telemetry/computer systems. The real-time processing capacity of these systems is much stronger than traditional telemetry systems, and they are being used in a growing range of applications. The main part of this article introduces the system architecture, software structure, real-time software functions, and design ideas for a programmable telemetry/computer system.

I. Introduction

Telemetry technologies are widely used in the aeronautical and space industries as well as in a wide range of applications in geological surveying, earthquake prediction, and other areas. Telemetry technologies have become an indispensable means of measurement in certain extremely toxic, highly radioactive, high temperature, or other dangerous environments. This is particularly true of the rapid growth of semiconductor integrated circuits and the computer sciences, which has profoundly affected the field of telemetry. Telemetry/computer systems created by integrating computers with telemetry systems have enormously improved the functions of early telemetry systems and given them a real-time telemetry data processing capacity.

In a typical computer system, all operations in the operating and control systems respond as needed to requests from users and other sources. When they are temporarily incapable of handling certain requests, the computer retains these requests until they are processed. In a telemetry computer system, however, the flow of telemetry data is created at a distant computer site and is not computer-controlled, so operation of a telemetry computer system during data

collection depends on the flow of telemetry data and operates by using time system signals to drive the entire system. Time system signals include bit, character, and frame synchronization signals. In PCM (pulse code modulation) telemetry systems, multichannel converters create a frame synchronization signal to instruct the computer to begin collecting telemetry data. At this time, the computer collects telemetry data according to the word synchronization frequency into the data buffer. When the multichannel converter creates another frame synchronization signal, it indicates that the previous frame of data has ended and a new frame of data has begun. Obviously, a measurement parameter can be missed if the computer fails to receive the word synchronization signal. The information in an entire frame can be lost if it fails to receive the frame synchronization signal. Thus, real-time work in the entire system is drive by an event--the obtaining of telemetry data. This is another difference between telemetry-computer systems and conventional computer systems.

The programmable telemetry/computer system we developed can be used for civilian telemetry and space flight instrument testing. This system now has been delivered to users and is receiving good evaluations from them.

II. Programmable Telemetry/Computer System Architecture

Basic functional characteristics of the telemetry/computer system:

1. Real-time capabilities. The computer must have a fast response capacity because operation of the entire telemetry/computer system is driven by an event, the acquisition of telemetry data. It must respond in real time to interrupt request signals arriving from different interrupt sources and process them in real time. For telemetry systems with high collection speeds, two adjacent pieces of telemetry data arriving together may be separated by only a few to tens of microseconds. If a microprocessor is used to control telemetry data collection, the controller will collect the telemetry data from the telemetry front end equipment into the buffer of the computer. This places very high demands on the computer and associated hardware.

2. Concurrent operation. When the telemetry/computer system collects data, it also must process concurrently the collected telemetry data in real time. Real-time data processing includes memory storage to record all telemetry data on a pre-inspection tape. Real-time processing also includes real-time analysis of the telemetry data, computation, display and printing, and so on.

The real-time and concurrent nature of the telemetry system places special demands on system architecture and software organization. It is obvious that a single microprocessor cannot concurrently process several types of real-time telemetry data. When designing our "programmable telemetry ground station," we chose the Intel 86/360 computer system, which has a high performance/price ratio. From the system architecture perspective, this is a multiprocessor system, with the multiprocessors cooperating under management by an operating system. These microprocessors can cooperate at different levels and inter-communication between microprocessors is possible. The architecture of the programmable telemetry/computer system is shown in Figure 1.
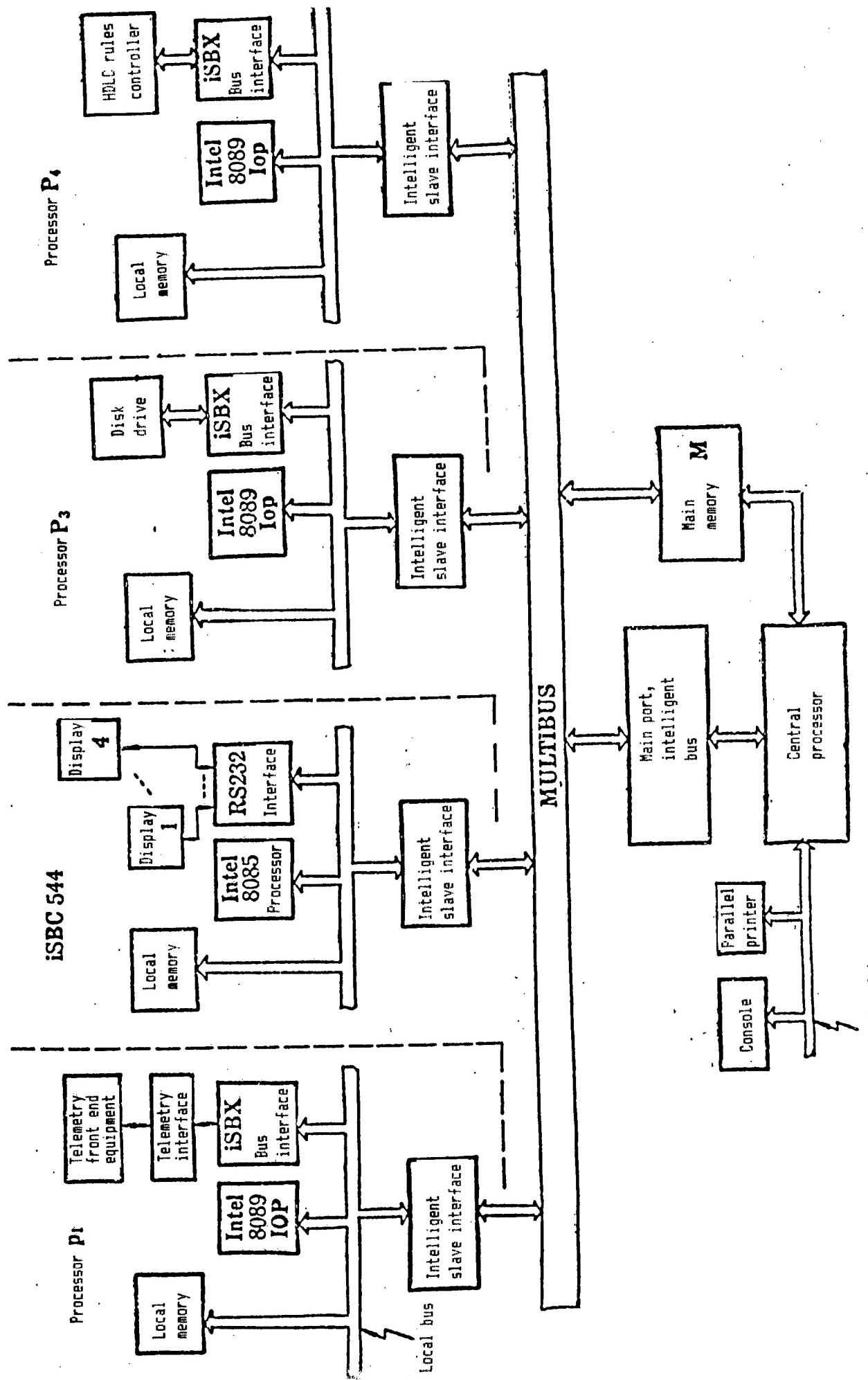
Figure 1. Programmable Telemetry Computer System Architecture

The computer subsystems in this computer system are composed of several single boards serving as basic cards which are connected to the same shared multi-processor bus--a Multibus. To reduce memory contention, each microprocessor has its own local memory. Thus, while each microprocessor is executing a program, it can select commands from its own local memory and process data in its local memory. It uses the bus to contact the main memory only when it exchanges signals with other microprocessors. The central processor has a special channel to the main memory. This eliminates delays in command execution due to memory tie-up by other processors when the central processor is fetching a command. The central processor controls the start and stop of each processor.

III. The Operating System of This System

The Intel 86/360 multiprocessor system in our programmable telemetry ground station has a flexible architecture, ease of expansion, and other positive aspects, and it is extremely well-suited to real-time applications. The iRMX86 real-time OS which manages the entire system also is an advanced technology embodying operating system developments in the 1980's. This real-time OS supports multitask concurrent operation and has an excellent interrupt processing system capable of handling up to 256 interrupt levels.

The iRMX86 is a goal-based open real-time OS. The OS-user interface is a group of system calls and a group of commands. Operators use system calls to achieve the desired operation. The iRMX86 real-time OS also provides the user with system calls for every operating system level from kernel and basic I/O system calls to general development interfaces and man-machine interface system calls. Thus, the user can use each level of the OS, with each level in the operating system being open to the user.

The open nature of the iRMX86 comes from the ability to cut out and embed this real-time OS. In real-time applications, software workers always want to be able to adapt the chosen real-time OS to their own special uses, and they want the shortest real-time system response. It is quite hard for OS designers to design a real-time OS adapted to all uses, however, so real-time application software workers hope to provide them with a real-time OS composed of several modules or subsystems to allow users to select modules or subsystems adapted to their own special purposes according to their needs. This means the ability to cut out and embed the real-time OS to allow the user to enter special purpose programs they have written into the system, with the chosen modules or subsystems forming a special purpose real-time OS adapted to the user's needs. The iRMX86 is a real-time OS which combines cut-out and embedding functions in one unit. It is composed of a kernel, basic I/O, expanded I/O, loader, general development interface, man-machine interface, and other subsystems. The user can employ the interactive configuration service program ICU provided by the system to select the required subsystems and add equipment and applications programs adapted to their own special uses. Then, the ICU automatically completes a new system configuration to create a special purpose real-time OS to meet the user's needs. In this way, the user does not have to worry about the details of real-time OS design but instead can work on designing software to handle actual applications.

IV. System Functions

The programmable telemetry ground station real-time applications software
system RTAS can perform the following functions:

1. It can set up telemetry front end equipment so that it obtains the desired
data format and works in the user-defined working state. The entire setup process
is menu-driven. Thus, a telemetry technician without programming experience
also can use the console to write setup commands. The setup information includes:
data word length, number of data words in a telemetry data frame, number of data
words in each frame, working state of the receiver and time coder, and so on.
After setup is completed, the configuration document is formed. Because the
system can store all configuration documents, the telemetry front and equipment
can be set up before it begins executing its tasks. It can also switch working
states and data formats in real time when actually executing tasks according to
the user-specified configuration document.

2. It collects the high speed telemetry data flow into a buffer. We used dual
buffers. The telemetry data fills up one buffer first, and when it is full,
begins to fill a second buffer. At the same time, the contents of the first
buffer are transferred to the main memory for real-time processing, thus
rotating between two buffers. The buffer size is fixed but the number of
telemetry data frames stored in each buffer changes with the data word length
and frame size.

3. It can record all telemetry data on tape in real time to permit later re-
loading for post-event data processing. All or part of the telemetry data is
stored in real time on disk.

4. It does real-time processing of the telemetry data. Real-time processing
includes real-time analysis of the telemetry data, and real-time display and
printing. Display and printing are done at the console and an iSBC544 processor
is used to manage up to four displays or parallel printers. The display can
be a Chinese cahracter terminal, remote intelligent terminal, or image display.
The original telemetry data can be displayed and printed according to user
needs, and it can display and print data which has undergone real-time
processing. For example, the original telemetry data can be converted into
engineering and physics quantities and displayed, or the results of processing
can be displayed in the form of line drawings. It can make over-limit checks
of certain measurement parameters during printing and display to provide
real-time warnings.

5. It has supporting HDLC [high-level data link control] data transfer, meaning
that it can transmit certain user-specified measurement parameters according to
HDLC rules from the programmable telemetry ground station to the control center.

V. Design Principles and Software Structure of the Real-Time Software

Because the iRMC86 is a goal-based, open real-time OS, it provides an excellent
environment for users to design and develop their own real-time applications

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software. We used the iRMX86 kernel, basic I/O, expanded I/O, general
development interface, and man-machine interface to enter a program we designed
to distinguish among various pre-defined events into the system via the ICU to
form a special purpose real-time OS for telemetry purposes. On this OS
foundation, we designed a programmable telemetry/computer system real-time
applications software RTAS.

The RTAS is a modular software system. It is easily revised and expanded, has
an excellent user interface, and can do post-event processing.

One principle we used in RTAS design was enabling the computer to do as much
work as possible. For this reason, we divided the overall system functions
into several tasks and made full use of the operating system's ability to
support multitasking and concurrent execution to keep each microprocessor busy
at all times. This achieved true concurrent operation of these microprocessors
and gave the telemetry system a rather strong real-time processing capacity.

Processor P_1 is responsible for managing the telemetry front and interface (see
Figure 1). It is based on an Intel 8089 IOP [input/output processor], has two
channels, and is capable of simultaneous execution of the programs in each
channel. Channel 1 is responsible for collecting telemetry data and time codes
in a DMA [direct memory access] format into the buffer and switching buffers
when the buffer is full. The work flow chart of channel 1 is shown in Figure 2.

Channel 2 is responsible for transmitting the data from its buffer into the main
buffer. We also opened two $\leq$ 64k-long buffers in the main memory. Each
telemetry data frame is $\leq$ 1024 + 3 time words long. Thus, the number of frames
each main buffer can hold $j(j = 0.1)$ varies, with the actual value being
determined by the user. The work flow chart of channel 2 is shown in Figure 3.

After the frame length has been specified, the larger the main buffer, the more
data frames it can hold. Because it can create an interrupt signal only after
channel 2 has filled the main buffer and then do the various types of real-time
processing, this processing actually is quasi-real-time processing. However,
if we make the buffer very small, as small as the length of each frame, each
data frame can be transmitted via channel 2 to the main memory after it is
collected and create an interrupt signal, after which it does real-time
processing. Obviously, because an interrupt is created after each frame of
data is transmitted to the main memory, a great deal of interrupt processing
greatly increases the expense of the operating system, which may reduce the
real-time processing capacity of the system. This is especially true for
systems which have a rather high collection speed. It is apparent that the
user should determine the size of the main buffer according to actual
requirements to increase system efficiency.

```
                    ┌─────────────────┐
                    │      Start      │
                    └─────────────────┘
                             │
          ┌──────────────────────────────────────┐
          │  Shift data port address into pointer │
          │  register GB, configure for DMA       │
          │  operation (external stop pattern)    │
          └──────────────────────────────────────┘
                             │
          ┌──────────────────────────────────────┐
          │  Shift local bufferi(i = 0,1) start   │
          │  address into pointer register GA     │
          └──────────────────────────────────────┘
                             │
          ┌──────────────────────────┐
          │  Wait for frame           │
          │  synchronization signal   │
          └──────────────────────────┘
                             │
          ┌──────────────────────────────────────┐
          │  Enter DMA cycle, collect telemetry   │
          │  data and time signals into local     │
          │  memory i at word synchronization     │
          │  frequency                            │
          └──────────────────────────────────────┘
                             │
          ┌──────────────────────────────────────┐
          │  When frame synchronization signal is │
          │  generated, this DMA cycle ends and   │
          │  the start address of local buffer    │
          │  i + 1 (mod2) is shifted to pointer   │
          │  register GA                          │
          └──────────────────────────────────────┘
```

Figure 2.   Channel 1 Work Flow Chart

Processor P_3 manages the disk drive. Because P_3 is configured into the original
system, the system-supplied write operations can provide memory storage of the
telemetry data. An iSBC 544 manages up to four displays and observes the
following rules in communicating with the central processor: unit base + 0 and
base + 1 are command word segments and state word segments. They provide a
communications interface between the central processor and iSBC 544. The base
is the base address of the iSBC 544 dual-port memory, and it is configured with
a jumper wire. After the iSBC 544 is prepared it configures the state word
segment in an "idle" state. When the task in the central processor checks this
indicator, it enables transmission of the telemetry data to the dual-port
memory in the iSBC 544, after which it writes the command to start the iSBC 544
into the command word segment (base + 0). Once the iSBC 544 begins working,
it first configures the state word segment into a "busy" indicator and then

displays the time codes and telemetry data on the four displays according to a specific management strategy. After the results are displayed, it again configures the state word segment into an "idle" indicator. If the task inquiry in the central processor inquires when this indicator is busy, the preceding frame is not displayed.
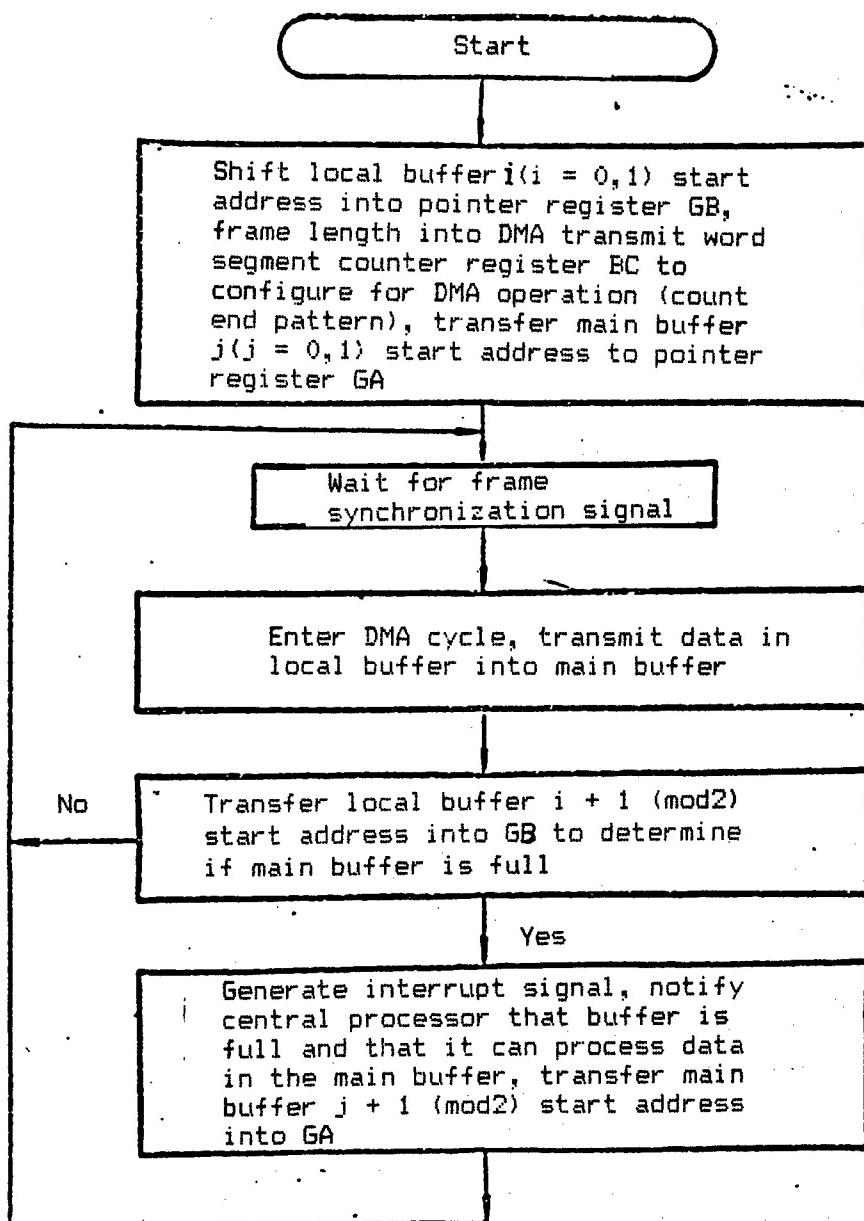
Figure 3. Channel 2 Work Flow Chart

Because the real-time application OS supports multitasking and concurrent operation, we do the following tasks in the central processor: monitoring and control tasks, real-time transfer and storage tasks, and display and printing tasks (if necessary, it also has data transmission tasks). When the system starts working it first enters the monitoring and control tasks and waits for the user to input the information required for real-time work. For example, do they require data transmission? Is it to be configured with the iSBC 544? What is the length of the main buffer? and so on. After the user inputs are finished, it initializes each of the processors and completes each task. Moreover, it holds these tasks and waits until a particular event occurs. Then it provides acceptable commands to the user in menu form. The system begins collecting data after the user inputs the collect command. When the main buffer is full, channel 2 generates an interrupt. After the corresponding interrupt processing program does this simple processing, it calls up each task. The real-time storage task stores the data in the buffer. The display and printing tasks process the telemetry data by frames. First it checks to see if the iSBC 544 is busy and starts the iSBC 544 if it is not. Otherwise, it does not display that frame of telemetry data. Next, it converts engineering and physics quantities, analyzes the data according to the various user-defined parameters, and displays or prints them. If data transmission is required, the data transmission tasks start P_4 for each specific time period. It also selects the data being transmitted into P_4's local memory and transmits it according to HDLC rules under control by P_4. After the user keys in the command to stop data collection, the monitoring and control tasks stop the work in each processor and cease data collection and real-time processing work.

VI. Factors Limiting System Performance Improvements

Because each processor has its own local memory in an Intel 86/360 multiprocessor architecture environment, command selection and data processing can be carried out in the local memory, which substantially reduces memory contention. However, each processor must use the bus to exchange information with other processors. Although the frequency/bandwidth ratio of the bus is much higher than the main memory, bus contention inevitably becomes a "bottleneck" to increasing overall system performance when the speed of data collection reaches a certain level. Moreover, because processor P_1 manages the telemetry front end interface and channel 1 collects the telemetry data into the local buffer, using software to switch buffers also restricts increases in data collection speeds. In the area of software, because the iRMX 86 real-time OS does not provide a concurrent program design language, it can only use the mechanisms provided by the operating system which support concurrent operation, such as signal lights, signal boxes, mail boxes, and so on for communication and synchronization of multiple tasks. This undoubtedly raises system expenses.

References

1. INTEL, INTEL 86/360 XILIE JIAOHUSHI XITONG PEIZHI ZHINAN [Guide to Intel and Intel 86/360 Series Intersystem Configuration], State-run Plant 574, 1985.

2. Wu Deyu [0702 1795 7183], "Lectures on Telemetry Technologies," YAOCE JISHU [TELEMETRY TECHNOLOGY], No 2, 1983.

3. O.J. Strock, Telemetry Computer System: An Introduction, pp 295-312, 1983.

4. K.B. Robert, A Generic Telemetry Host Computers Software Architecture, I.F.T., pp 491-500, 1985.

5. Gale Williamson,"User Programmable Real-Time Processing in Modern Telemetry Systems," pp 483-490, 1985.

China's HN-5 Shoulder-Fired Missile System Described

[Article by Xiao Lin [5618 2651] and Jiang Yuetie [5592 6390 6993]]

[Text] Abstract: HN-5 is China's first domestically-made man-portable air defense missile system capable of making tail-on engagements against jets or head-on engagements against propeller-driven aircraft and helicopters under visual aiming conditions. This article describes the HN-5 missile system in detail.

Introduction

Utility

The HN-5 weapon system is a portable one-man shoulder-launched extreme low altitude anti-aircraft missile system. It is powerful weapon system for direct cover of mechanized foot soldiers, tanks and paratroopers. Combined with other anti-aircraft weapons, it can also be used in the defense of strategic targets.

The HN-5 is easy to use, maintain, and store. It has good ground mobility and can be carried with a shoulder strap. In combat, it is fired on the shoulder while standing or on one knee.

The HN-5 missile can be used whenever the visibility is adequate. The user may freely choose a launch site or move from one site to another. Launchings can be made whenever the target is spotted visually and the user is not in danger of being attacked. It can be launched in open fields, in trenches, on water, in swamps, on rooftops, or from vehicles running on level ground. Once the missile is launched, it automatically tracks the target and no more operations are required of the launcher, hence allowing the launcher to take cover or move away.

The missile attacks jets by tailing and attacks propeller-driven aircraft or helicopters from the front or rear.

Components

The HN-5 missile system consists of the missile, the launcher tube, the launching mechanism and the ground battery set.

1. Missile--The missile is loaded in the launcher tube; the front and aft guidance rings match the inner wall of the tube and the missile is positioned properly with the aid of a stop on the launcher tube. When the missile is in the tube, its rudder and tail fins are folded against the tube wall; once the missile is out of the launcher tube, the rudder and tail fins spring open.

2. Launcher tube--The launcher tube is a carrier and container for the missile when it is transported, it is used in aiming and launching the missile, and it also serves as a shield for the soldier against burns by the hot gas stream from the motor.

The body of the launcher tube is made of fiberglass. Mechanical aim and preset indicators are installed on the outer surface of the tube. After firing a missile, the launcher tube may be returned to service following inspection.

3. Launching mechanism--The launching mechanism readies the missile for firing and controls the firing process. When a target is intercepted and a target signal of sufficient strength is detected, the launching mechanism emits acoustic and optical signals indicating that the missile is ready for launching. The operator then pulls the trigger and fires the missile. Depending on the magnitude of the line-of-sight angular velocity of the target, the missile may be fired manually or automatically. The launching mechanism analyzes the tracking signal and activates the full launching sequence when the launching conditions are met. The motor is then started and the missile is launched. The launching mechanism may be used repeatedly.

4. Ground battery set--The battery is a one-time use thermal battery. It provides electrical power to the various components of the missile system in readying and launching the missile. The battery is small, light, and has a fast response and a large capacity. It is also easy to carry and requires no maintenance.
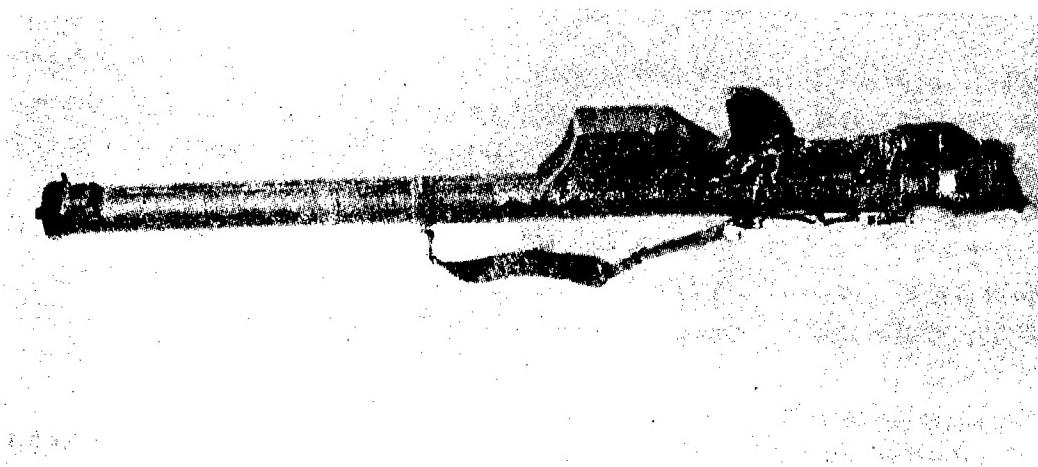
Figure 3. The HN-5 missile weapon system

The principal tactical and technical specifications of the system are shown in the table below.

Table 1. Principal specifications

Total weight	15 kg
Length	1508 mm
Weight of missile	9.8 kg
Length of missile	1423 mm
Diameter of missile	72 mm
Maximum kill range	4200 m
Maximum kill altitude	2300 m
Guidance method	Proportional approach
Guidance mechanism	Passive IR automatic homing
Temperature range for operation	-40°C to +50°C

The Missile

1. Outer profile and aerodynamic configuration

The outer profile of the HN-5 missile is a slender body with a blunt nose. The spherical crown of the head joins with a truncated cone and the body is cylindrical. A pair of rudders is located at the front portion of the missile and two pairs of tail fins are at the rear end of the body to form an "x"-shaped "canard" configuration.

Figure 4. The HN-5 missile

2. Sectional design

For the ease of manufacture, assembly and exchange of components, the missile is made into four sections that are then assembled together with adjoining bolts. From the head to the tail, the four sections are the guidance head section, the rudder mechanism section, the warhead section, and the motor section.

a. Guidance head section

This section contains the coordinate tracking device and the electronics compartment. The coordinate tracking device is a gyro tracking device with infrared optics and the electronics compartment consists of the guidance head tracking circuit and the missile automatic pilot circuit.

The radiation from the infrared source of the target is received by the coordinate tracking optical system. After focusing, the radiation is projected onto the reticle and the infrared detector. The relative deviation between the optical axis of the coordinate device and the line of sight to the target determines the location of the projection on the reticle. The electrical pulse output from the infrared detector therefore contains the magnitude and orientation of the deviation.

Signals are processed in the electronics compartment and become in one channel the control signal for the rudder and in the other channel (entering the precession coil of the coordinate tracking device) the control signal for the precession torque of the gyro rotor. Since the main reflecting mirror of the optical system is on the front end of the rotor, the axis of the rotor is also the axis of the optical system. The precession of the gyro is therefore also the tracking process that eliminates the relative deviation of the optical axis of the coordinate tracking device.

b. Rudder mechanism section

This section contains the "canard" aerodynamic configuration rudder--the movable front fins. The gas turbine that controls the rudder surface and the gas generator are located in this section. This section also contains a gas turbine electrical generator that supplies power to the missile and a rectifier and voltage regulator. Also housed in this section are transducers and demodulators that sense the angular velocity of the missile.

c. Warhead section

This section is divided into the warhead and the detonator. The warhead's main functions are to kill by concentrated energy, fragmentation and explosion. The detonator is electro-mechanical fully secured trigger detonator. If the missile missed the target, the self-destruction device of the detonator will cause the missile to explode.

d. Motor section

This section consists of the launch motor (with ignitor), the main motor (with delayed ignitor) and the tail fins of the missile. The launch motor is used to launch the missile by giving the missile the necessary muzzle velocity. Since the launch nozzle is tilted, the missile also acquires a certain angular velocity about its axis. This is needed in the single channel control system with a pair of rudder surfaces.

The main motor is a single chamber dual thrust solid fuel rocket motor. The first thrust accelerates the missile to attain the maximum velocity and the second thrust then maintains this velocity.

The delayed ignition assures that the charge in the main motor is ignited after the missile has left the launch tube for at least 5.5 meters. This is for the safety of the launcher.

The tail fin assembly consists of four fins and the spring mechanism that releases the fins. The functions of the tail fins include providing lift and maintaining static stability. Also, since the plane of the tail fins is tilted relative to the axial plane of the missile, the missile continually rotates about its axis in flight.

Characteristics of the Control System

Because the missile rotates about its axis during flight, the control method is different from a missile that does not rotate. First, the rotation solved the tilt stability problem. Second, the rotation also eliminated the distinction between the conventional pitch and yaw. As described above, the missile has only one pair of rudder surfaces to control the deflection in concert with the rotation of the body. This arrangement allows dynamic movement in any direction in the horizontal plane.

Since the gas turbine motor of the missile has a powerful thrust and a fast response, it is suitable for relay movements. One of the unique features of the control system is that the rudder deflection is controlled by pulse width. The rudder has only two resting positions at the extremes of the positive and negative deflection angles. The transition between the two positions is done almost instantaneously. In coordination with the rotation of the body, the time of transition can also be controlled, that is, the time of stay in either extreme positions can be controlled. An important advantage of this control scheme is that the rudder is an open circuit execution element and requires no rudder position feedback signals.

Since there is only one pair of rudder surfaces, a single channel automatic pilot is sufficient for the transmission and process of the signals. The control signal from the guidance head has a sine wave form with a period equal to the rotation period of the coordinate tracking gyro, and its amplitude and phase correspond respectively to the magnitude and direction of the line-of-sight angular velocity. The autopilot changes the signal

into pulse width waveform that can be used in controlling the rudder. The period is the same as that of the missile rotation and the line-of-sight angular velocity information is contained in the width of the positive and negative pulses and the time of transition.

The damping channel of the autopilot uses the angular velocity transducer as a sensor to achieve the damping of angular oscillations about the transverse axis of the body.

In summary, the control system of the missile makes use of the axial rotation of the body and has a single channel circuit. The circuit and components are therefore very compact and are ideal for the small volume and light weight of the missile.

Production and Application

The HN-5 weapon system has not only met the various flight test specifications, it has also passed the tests for usage under extreme environmental conditions. On 1 October 1984, in the review of troops celebrating the 35th national day of the People's Republic, HN-5 missiles were reviewed by our party and state leaders.

After finalizing the design, the HN-5 system was mass produced. The quality of the product has been stable and reliable and all the tests were successful in the first trial. In 1987, HN-5 was declared a high quality product of the nation.

An integrated tester for testing HN-5 has also been developed.

In order for troops to use this weapon system, a complete set of simulation training equipment has been designed and manufactured. Trial use by the services proved that the equipment is practical and easy to use and the training procedures are effective.

Output Characteristics of Large Aperture Nd:YAG Disk Laser*

[Article by Cao Weilou [2580 3262 2869], Zhang Meizhen [1728 2734 3791] and Hua Xuelei [5478 7185 5623] of Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences: "Characteristics of a 40mm Aperture Nd:YAG Disk Laser"]

[Text] Abstract

The paper reports the characteristics of a 40mm aperture Nd:YAG disk laser for the first time. Theoretical analysis indicates that this kind of device can produce a mean power of the order of 10^3 Watts.

I. Effect of on Parasitic Oscillation on Laser Output Characteristics

In the system we studied, the power limiting area of the Nd:YAG disk is one order of magnitude higher than that of a thick plate or rod device.[1,2] Therefore, it has the potential to be developed into a high-frequency megawatt or continuous kilowatt laser device. However, if the laser propagates along the short axis, the parasitic oscillation in the long axis direction often would consume all the energy stored in the disk to seriously affect the laser output when the gain reaches a certain level.[3] Hence, parasitic oscillation control is a key problem in the development of a high-power disk laser. The usual method is to control the laser gain in the disk and to eliminate the reflection and scatter on the side. The condition for parasitic oscillation to occur is

$$R \cdot \exp[n(g_0 - r)l] \geqslant 1_o \qquad (1)$$

where R is the reflection or scatter on the side, 1 is the length of the long axis of the Nd:YAG disk, n is the index of fraction, r is the dynamic absorption coefficient and g_O is the laser gain index.

Once the shape and size of the disk are defined, parasitic oscillation is only dependent on the gain of the laser medium and its reflection and scatter characteristics. From (1), the threshold gain to produce parasitic oscillation is

$$g_{oth} - r = \frac{-\ln R}{nl},\qquad (2)$$

When the side is ground dull, we get

$$R = \left(\frac{n-1}{n+1}\right)^2.$$

The net gain index is

$$g_{oth} - r = \frac{2\ln\left(\frac{n+1}{n-1}\right)}{nl}.\qquad (3)$$

Under these conditions, $g_{oth} - r$ is 0.17cm^{-1}. This means that when the net gain index of the laser reaches 0.17cm^{-1}, its output will be affected by parasitic oscillation. The gain begins to saturate and the efficiency drops.

II. Calculation of Maximum Mean Laser Power Output

Since the goal of the device is to obtain the maximum mean laser power output, it is necessary to calculate the potential output power of the device under design. The system is uniformly pumped and surface cooled. Therefore, there is an axial temperature gradient. This eliminates the thermal lens effect and thermal double refraction effect in the system. The power of this device is limited by the thermal cracking stress of Nd: YAG, which is 2000W/cm^2.

Based on a thermal stress analysis of the disk[4], we have

$$\sigma_{xx} = \sigma_{yy} = \frac{2\alpha E}{3(1-\nu)}\Delta T,\qquad (4)$$

$$\Delta T = \frac{Qt^2}{8K}.\qquad (5)$$

where σ_{zz} and σ_{yy} are the surface stress on the disk, ΔT is the temperature difference between the center and surface of the disk, Q is the thermal power density absorbed by the disk, t is the thickness of the disk, K is the thermal conductivity of Nd:YAG, α is the linear expansion coefficient, E is the Young's modulus and ν is the Poisson's ratio.

Let us define M_S as the quality factor of the material:

$$M_s = \frac{(1-\nu)K}{\alpha E}.$$ (6)

We get

$$\sigma_{zz} = \sigma_{yy} = \frac{Qt^2}{12} \cdot \frac{1}{M_s},$$

Let us define R_S as the thermal stress endurance factor, then

$$R_s = \sigma_s M_s = \frac{Q_s t^2}{12}.$$ (7)

where σ_S is the thermal cracking stress and Q_S is the limiting thermal power density.

At this time, the limiting mean thermal pumping power density and limiting mean thermal endurance power are:

$$Q_s = \frac{12R_s}{t^2},$$ (8)

$$P_a = Q_s \cdot V = 12R_s\left(\frac{\omega L}{t}\right).$$ (9)

where V is the volume of the disk, and ω and L are the width and length of the disk, respectively. In the Nd-doped laser four-energy-level system, the thermal energy in the laser material is generally twice that of the energy stored and the efficiency to access the stored energy is only approximately 0.5. Thus, the maximum mean power of the laser is:

$$P_{laser}^{AVE} = 3R_s\left(\frac{\omega L}{t}\right).$$ (10)

For Nd:YAG, the quality factor M_S is approximately 4×10^{-5} WM/kg, $\sigma_s = 2000$ kg/cm^2, $R_s = 800$ W/m, therefore, for a 80x40x6mm^3 disk $Q_S = 267$W/cm^3, $P_a = 5070$W and $P_{laser}^{AVE} = 1267$W. For a laser device with two Nd:YAG disks, the maximum power output is 2500W. In the experiment, if we choose a safety factor of 5, it is hopeful to get 500W

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of average laser output. P-doped glass laser devices are one order of magnitude longer than that of Nd:YAG (see Table 1).

Table 1 Thermal Properties of The Laser Material & Maximum Laser Output

	n	ν	k W/m°C	α 10^{-6}/°C	E kg/mm²	σ_s kg/mm²	R_s W/m	P_a (80×40×6 mm slab) W	P_{laser}^{avg} W
N$_{0230}$ Q-100	1.54	0.24	0.82	9.60	7150	6.5	59	378	94
Quartz	1.45	0.17	1.38	0.55	7190	5.0	1450		
YAG	1.81	0.30	13.0	7.80	31725	20.0	790	4990	1247
GSGG	1.92	0.15	8.0	7.80	21000	17.0	700	4410	1102
LiYF$_4$	1.45	0.33	6.0	10.0	7650	3.4	180	1150	287

III. Experimental Study of the Laser's Characteristics

The experimental set-up is shown in Figure 1. Two 6x40x80mm³ Nd:YAG disks are placed at the Brewster angle. Except the two light passing surfaces, other sides are ground dull. A Model PT-1 laser energy meter was used to measure the power output of the laser. The results are as follows:
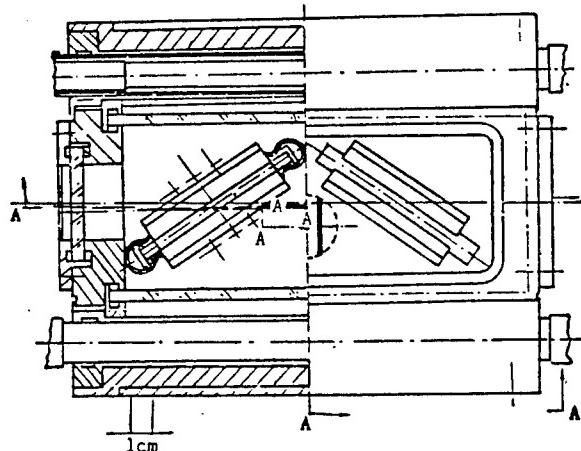
Fig. 1 Diagram of The Nd:YAG Disk Laser

1. Comparison of Laser Resonators

We used parallel F-P and spherical stable resonators for the Nd:YAG
laser and compared their laser output efficiency. Since the Nd:YAG
disks are placed at the Brewster angle and the mode volume of the
parallel F-P resonator is higher, it appears more appropriate to use the
parallel F-P resonator. However, the stable resonator is less sensitive
to maladjustment. It is easier to obtain a stable laser output. Figure
2 shows the experimental laser efficiency curves obtained with these two
resonators. The spherical resonator is obviously more superior to the
parallel F-P resonator.
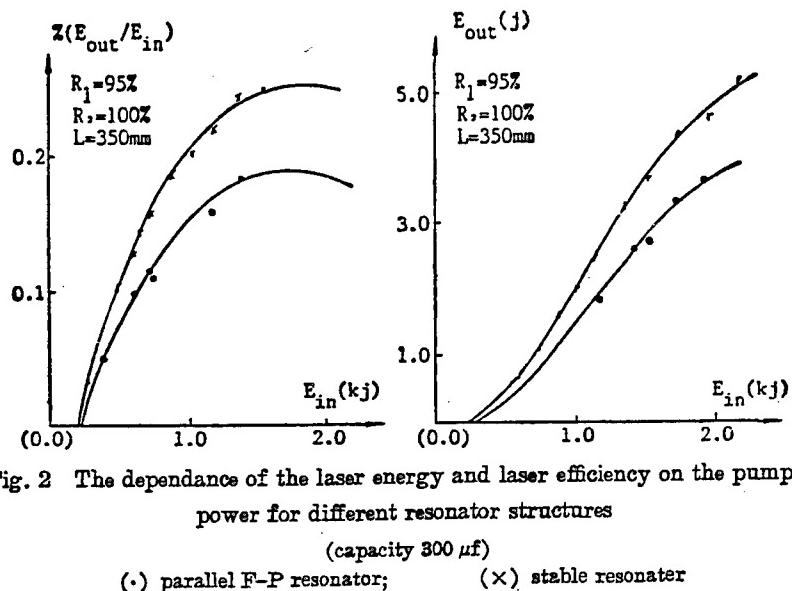
Fig. 2 The dependance of the laser energy and laser efficiency on the pumping
power for different resonator structures

(capacity 300 μf)

(·) parallel F-P resonator;   (×) stable resonater
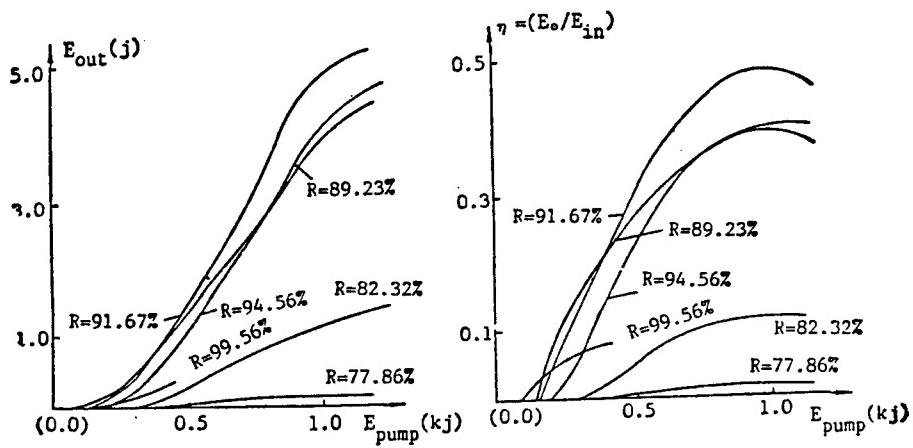
Fig. 3 Laser output energy and laser efficiency vs pumping power density

(R—reflectivity of coupling mirror)

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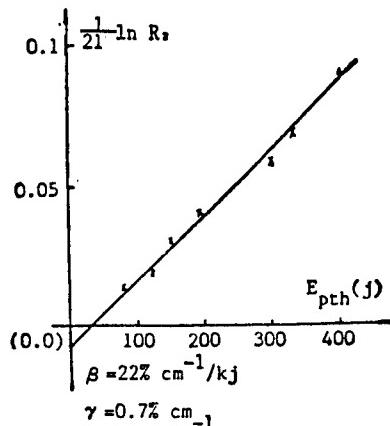
Fig. 4 Small signal gain coefficient vs pumping power density for the Nd:YAG disk oscillator

2. Optimal Coupling

We used different reflectance output mirrors to measure the laser energy output and to calculate the laser efficiency. The results are shown in Figure 3. Figure 3 shows that maximum laser efficiency is reached when the reflectance of the output mirror is 91 percent. In addition, we used the laser threshold method to measure the dynamic absorption coefficient of Nd:YAG, γ, which was found to be less than $0.8\ \%cm^{-1}$. The gain of a unit of optically pumped energy β is $22\%kJ^{-1}cm^{-1}$. The results are shown in Figure 4.

3. Focusing Structure

In order to optimize the optical pumping efficiency, we compared various focusing cavities. It was experimentally determined that the plane reflective focusing cavity is the best. Its maximum efficiency is 0.6 percent. The single pulse laser output energy exceeds 6J (see Figure 5).
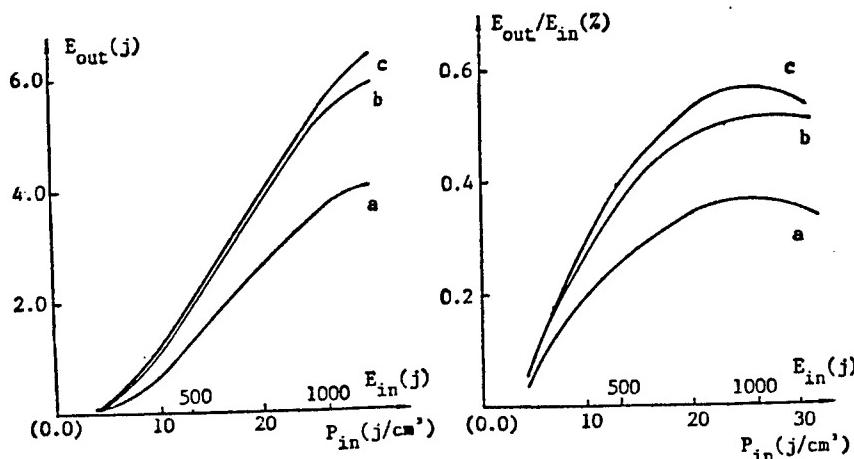
Fig. 5 Laser output vs pumping power for different pumping cavities
a) Parabolid   b) sawtooth   c) close coupling

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4. Parasitic Oscillation

In all the laser efficiency curves, we found that the efficiency begins to drop when the pumping energy density exceeds $27J/cm^3$. This indicates that parasitic oscillation along the long axis of the Nd:YAG disk has already seriously affected the laser system. The small signal gain coefficient is $22\%cm^{-1}$. Its product with the length of the long axis is only 1.8, which is consistent with the theoretical estimation described earlier. If the crystal is wrapped with a layer of infrared absorbing material to reduce or eliminate parasitic oscillation, high energy and efficiency can be obtained.

5. Pumping Homogeneity

We also used the laser threshold method to measure the pumping homogeneity of the lens laser. With 80 percent of the output volume, $\Delta\beta/\beta<10\%$. This shows that the disk laser is homogeneously illuminated.

6. Laser Amplification Characteristics

The Nd:YAG disk laser system was used as a laser amplifier and its laser gain characteristics were measured. This gain curve (see Figure 6) is in total agreement with that obtained with the oscillation threshold method. From the figure we see that parasitic oscillation occurred and the gain coefficient curve reached saturation when the pumping power density got up to $22J/cm^3$.
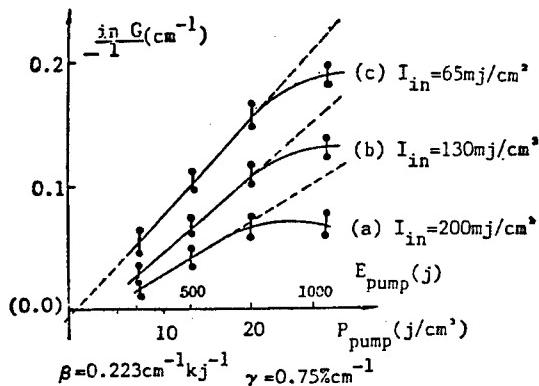
Fig. 6 Small signal gain coefficient vs pumping power density for the Nd:YAG disk amplifier

7. Interaction between Intense Laser and Defects in Nd:YAG Disk and Ultraviolet Filters

We tested the characteristics of the Nd:YAG disk before and after use. Particularly, the interaction between the strong laser and crystal defects. No apparent changes were found in crystal characteristics after six months of use. In order to prevent the Nd:YAG disk from forming a color center due to optical pumping, we used the filter developed by out institute. Spectroscopic analysis of a Nd:YAG disk used for six months showed no color center. The filter is effective.

IV. Conclusions

A laser was made with a large Nd:YAG disk prepared by a temperature gradient method.[4,5]. When the sides are ground dull, a non-saturated gain of $0.17 \ \mathrm{cm}^{-1}$ was experimentally determined. The energy per pulse for each 40 diameter beam is 6J. In theory, a laser system composed of 4 to 6 Nd:YAG disks can be expected to deliver an average power of over 1000W.

References

1. Wei-lou Cao, et al., Optical News, 1986 12, No. 9, 208.

2. T. J. Kane, et al.; Digest of CLEO, (1982).

3. Zheng Yuxia [6774 3768 7209] et al.; ACTA OPTICA SINICA 1986, 6, No. 4 (Apr), 381.

4. J. M. Eggleston; Opt. Lett., 1982, 7, 405.

5. Zhou Yongzong et al.; Journal of the Chinese Silicate Society 1983, 11, 375.

6. Zhang Meizhen et al.; Chinese Journal of Lasers 1984, 11, No. 11 (Nov), 665, Applied Laser, 1986, 6, No. 6 (Dec), 256.

Hardening Treatment for the Surface of 50 SiMnP Steel for High Power Laser

[Article by Yu Hongtao]0060 3163 3447] and Cao Tianshun [2580 1131 7311]
of Angang College of Technology]

[Abstract] The article proposes a method of phase-change hardening treat-
ment of 50 SiMnP rail steel by using a high-power CW CO_2 laser, so as to
form a 0.5 to 1.0 mm hardening coating (highly diffused martensite texture)
on the surface of heavy rail steel to increase its abrasion resistance
and to prolong its service life. The treatment is also advantageous in
enhancing anti-corrosion property and raising fatigue strength.

Five figures show the light path in an experimental installation, a harden-
ing curve of a thoroughly tempered layer, and metallographs of test specimens.
Four tables list data of chemical constituents of 50 SiMnP, its main tech-
nical parameters, as well as HRc and Hv hardness measurement ranges of a
specimen surface.

Characteristics of Electromagnetic Radiation Produced by Copper Vapor Laser

[Article by Wang Jinyue [3769 6855 2588], Xu Pinfang [1776 0756 5364], Xu Xiangdong [1776 0686 2639], Shen Tingting [3088 1250 1250], Luo Wanxiang [5012 5502 6272] and Xie Yu [6200 6877] of Number 605 Tianjin Institute]

[Abstract] With a narrow pulse of excitation current and high discharge current during its operation, a copper-vapor laser generates wide-band high electromagnetic radiation, which causes injury to the human body and interference to nearby electronic instruments. Therefore, it is necessary to study the electromagnetic radiation characteristics of the copper-vapor laser for shielding protection.

Four figures show a frequency spectrum for the discharge current, the relationship between radiation field intensity versus the product of laser pulse repetition frequency and discharge voltage, the relationship between radiation field intensity and transmission distance, and the frequency spectrum distribution of radiation field. References: 2, in Chinese. The article was received for publication on 23 November 1987.

Stimulated Raman Scattering, Stimulated Collision-Induced Fluorescence in Barium Vapor

[English abstract of article by Huo Yunsheng [7202 5366 3932], et al., of Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences]

[Text] The stimulated collision-induced fluorescence (SCF) at wavelengths of 472.6, 582.6 and 648.3 nm, as well as Raman-shifted radiation at 475 nm, were observed in the barium vapor pumped by a broadband pulse of the XeCl laser. The SCF was found to be self-terminated short pulses with FWHM of 3 ns, and its intensity increased with buffer-gas pressure. A time delay of as long as 20 ns existed between the front edges of the pump pulse and the Raman-shifted pulse, and the Raman conversion efficiency decreased significantly as the buffer-gas pressure increased.
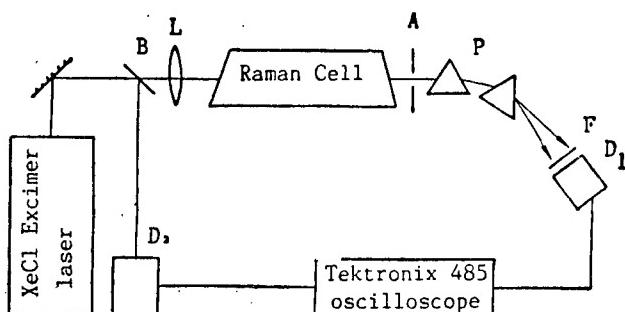
Schematic Diagram of the Experimental Arrangement.
B-beam splitter; L-lens; A-diaphragm; F-filter; D_1, D_2-photodiodes

References

[1] D. G. Cunnigham et al.; *Optica Acta*, 1986, **33**, No. 4 (Apr), 425.

[2] D. G. Cunningham et al.; *Optica Acta*, 1984, **31**, No. 3 (Mar), 249.

[3] M. G. Raymer et al.; *Phys. Rev. Lett.*, 1977, **39**, No. 21 (Nov),1326.

[4] R. Burnham et al.; *Opt. Lett.*, 1978, **3**, No. 6 (Dec), 215.

[5] D. Cotter et al.; *Opt. Commun.*,1978, **26**, No. 2 (Aug), 251.

[6] J. L. Carlsten et al.; *Phys. Rev. A*, 1977, **15**, No. 3 (Mar), 1029.

[7] C. E. Moore; "*Atomic Energy Levels*", 1971, **3**, (NSRDS–NBS 35, U. S. Government Printing Office, Washington, D. C.), 131.

[8] 霍芸生等; 《光学学报》,1986, **6**, No. 10 (Oct), 880.

[9] Y. Prior et al.; *Phys. Rev. A*, 1986, **33**, No. 4 (Apr), 2362.

[10] A. Yariv; "*Quantum Electronics*", (John Wiley & Sons. In., New York, London, Sydney, Toronto, 1975), 477.

Mechanical Rotating Mirror Isolator

40090019b Shanghai GUANGXUE XUEBAO [ACTA OPTICA SINICA] in Chinese Vol 8 No 8,
Aug 88 pp 722-726

[English abstract of article by Zhou Feng [0719 3536], et al., of Shanghai
Institute of Optics and Fine Mechanics, Chinese Academy of Sciences; Hu Shaoyi
[5770 4801 5902] of CITIC-Jiading Opto-Electronic Co., Ltd., Shanghai]

[Text] A new isolator--the high-speed mechanical rotating mirror isolator for
high power laser-fusion systems--is described in this paper. The isolator has
a high isolating ratio and a low insert loss, and it can also act as a spatial
filter and a backward laser beam isolator. The problem of synchronization is
investigated in detail. Our experiment shows that an isolator with a beam
aperture of 40 mm and a rotative velocity of 6000 rpm can achieve a timing
accuracy of better than 0.3 µs and a restoring accuracy of better than 1".
The factors affecting the accuracy are analyzed, and methods for improvement
are given.
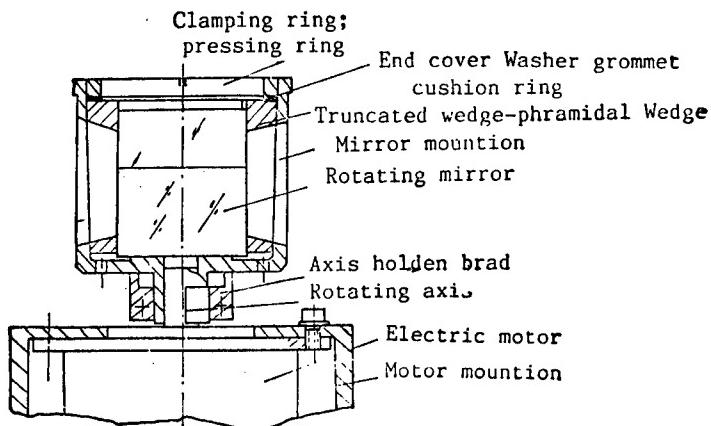
Structure of Rotating Mirror

References

1. Kuizenga, D.J., OPTICS COMMUN, Vol 22 No 2, August 1977 pp 156-160.

Network Synthesis of Microwave FET Ultra-Broadband Amplifier

[Article by Li Zhengfan [2621 1767 1581] of the Shanghai Jiaotong University Electronics Engineering Department; manuscript received 15 April 1987]

[Text] Abstract

This article describes theory and design methods for an FET ultra-broadband amplifier with a 100:1 frequency coverage. We began with a simplified model of FET microwave small signals and, with a precondition of providing the maximum gain bandwidth product, we did network synthesis using voltage transmission coefficient functions for an interstage network and then used optimization methods to readjust circuit parameters. The result was an amplifier with a flat gain response and low input/output SWR over an ultra-broadband range. Theory and design methods suggested in this article have been confirmed by experimental results.

I. Introduction

The use of resistance-matched networks to make two-octave microwave broad-band amplifiers now is maturing both in design theory and product development, and there have been reports from China in this area. One application now being suggested is to broaden the frequency band of microwave FET amplifiers to give them a frequency coverage in excess of 100:1, leading to so-called ultra-broadband amplifiers. They could be valuable in a broad range of applications in microwave instruments, electronic countermeasures, satellite communications, high speed data communications, and microwave time domain systems.

Several methods have been suggested in foreign countries for making ultra-broadband amplifiers. The main ones are traveling-wave amplifiers, direct-coupled amplifiers, feedback amplifiers, lossy matching network amplifiers, and so on. The first two are suitable for use in MMIC [monolithic microwave integrated circuit] patterns, but we will be hard put to use them in a wide range of applications when key problems persist with China's monolithic integrated circuits (MIC). Moreover, feedback amplifiers require microwave FET's with high transconductance values (usually with a G_m of more than 60

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to 70 mA/V) for optimum performance. However, when noise index require-
ments are not very high, amplifier circuits with lossy matching patterns
have rather simple configurations and place only the usual demands on
components. In addition, they can be made using a MIC pattern, which is a
more acceptable ultra-broadband amplifier program.

A lossy matching amplifier has a suitable compensating resistive component
added to a matching network to flatten its frequency response charac-
teristics. It also can directly provide low input and output SWR using
resistance matching over an ultra-broadband range. Kazuhiko Honjo et al.
proposed lossy matching in microwave ultra-broadband amplifiers some time
ago[1], but it actually was just a circuit program. There are no systematic
theory and design programs, and only optimized determined circuit parameters
are recorded. Although supplemented by later articles[2,3], it remains in a
semi-experimental, semi-chance state. This is particularly true of the
inability to determine the relationship between component parameters and
amplifier characteristics, making it impossible to use component charac-
teristics to predict amplifier indices.

This article suggests a circuit structure and corresponding network synthesis
methods for an R-∞ low-pass network ultra-broadband amplifier. This struc-
ture can fully exploit the gain bandwidth potential of FET devices. It can
provide an amplifier with the maximum gain bandwidth product for a given
component, and network synthesis can be used to derive interstage network
parameters on the basis of described component parameters and amplifier
frequency width requirements. This achieves optimal amplifier design, and
the use of network synthesis during the design process can achieve system
goal design.

II. Basic Theory

Because the frequency band coverage of ultra-broadband amplifiers may already
exceed 100:1, they can no longer be seen as the usual band-pass broadband
amplifiers but instead should be viewed as low-pass amplifiers which have
their low-end frequency restricted by current isolation and high frequency
short-circuit capacitances.

As everyone knows, the unidirectional simplified equivalent circuit in
Figure 1(a) can be derived after small signal S parameter fitting of a
microwave FET. When used as an ultra-broadband amplifier, it usually satis-
fies the relationship $\dfrac{1}{\omega_{max}C_i} >> R_i$ at a high-end frequency f_{max} to assure
amplifier gain. Here, we can ignore R_i in the model and convert it to the
model shown in Figure 1(b). This model illustrates two points:

1. Microwave FET's are grid voltage controlled current sources. Their
transconductance G_m can be approximated as not changing with frequency.
Thus, we can assure that the overall amplifier response is flat merely by
giving the amplifier's interstage networks a flat voltage transmission
response over a broadband range.

2. The input and output of a microwave FET are both parallel capacitance components, so they can be used precisely as front-end and tail-end components in low-pass interstage networks, and they have an ultra-broadband response.


(a)

(b)
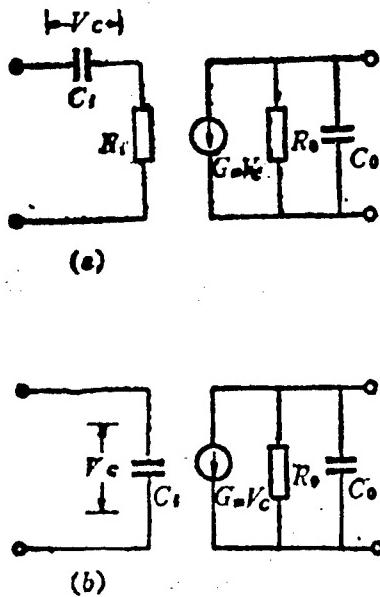
Figure 1. Simplified Model of Microwave FET

The component and interstage network stage connection pattern is shown in Figure 2. C_{o1} and C_{12} are, respectively, the output and input capacitances ahead of and following the component. They are absorbed by interstage network M to form the whole network M', making it a low-pass type.
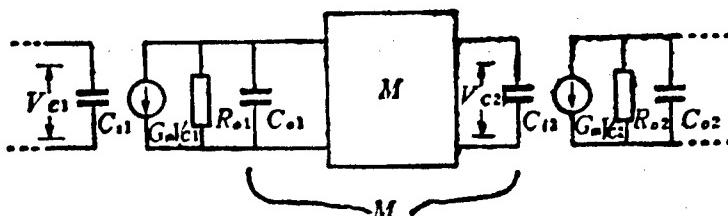
Figure 2. Stage Connection of Microwave FET Device and Interstage Network

The key lies in what type of low-pass network to use to most effectively exploit the gain broadband potential of the FET device. Usually, a resistance is connected on either side of a low-pass network, so it can also be called an R-R low-pass network. By changing the current source at the left of Figure 2 into a voltage source, R_{o1} serves as a terminal resistance or signal source internal resistance at the left side. The resistance R_2 can be added in parallel with C_{12} on the right side to serve as a terminal resistance or load resistance. After normalization, this type of R-R network is a conventional low-pass prototype filter.

Analysis shows that the above R-R network does not fully exploit component parameters. In contrast, our R-∞ low-pass network structure has a greater gain bandwidth utilization ability than the previous one, so we will begin with quantitative analysis of it. Figures 3(a) and 3(b) are, respectively, R-R and R-∞ low-pass interstage networks. The input end has been converted into a voltage source, and we assume that the voltage transmission coefficient $K = \dfrac{V}{E}$ is a function of ω. K should have the maximum gain bandwidth product given component parameters, input/output capacitances, and other conditions, to assure that the amplifier as a whole has a maximum gain bandwidth product. Bode[4], Fano, Youla[5], and others have offered theories on the gain bandwidth of resistive networks, but this gain refers to power transmission coefficients and is only appropriate for R-R networks. We came up with a way to use R-R networks indirectly to derive the gain bandwidth limiting condition for R-∞ networks, and we used similar gain bandwidth conditions represented by voltage transmission function K to compare the two types of networks.
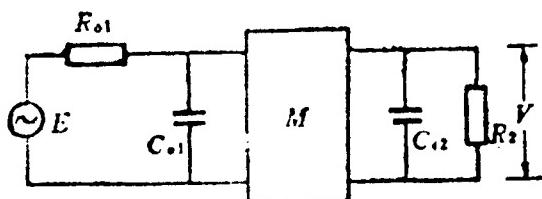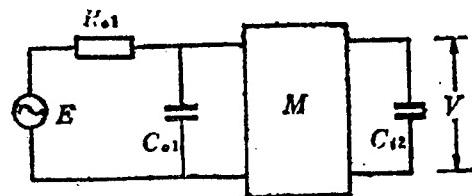
(a) R-R Network   (b) R-∞ Network

Figure 3. R-R Network and R-∞ Network

In the R-R network in Figure 3(a), we can use the Bode formula to extrapolate the gain bandwidth limiting condition for the R_L and C_{12} parallel load as

$$\int_0^\infty \ln \frac{1}{1 - |S_{21}(j\omega)|^2} \, d\omega \leqslant \frac{2\pi}{R_L C_{12}} \tag{1}$$

By definition we have

$$|S_{21}|^2 = \frac{|V|^2/R_L}{|E|^2/4R_{p1}} = \left|\frac{V}{E}\right|^2 \cdot \frac{4R_{o1}}{R_L} = |K|^2 \cdot \frac{4R_{o1}}{R_L} \tag{2}$$

Substituting formula (2) into formula (1), and setting $R_L \to \infty$ as the assumed extreme limit, we derive the gain bandwidth limiting condition for R-∞ network voltage transmission coefficient K.

$$\lim_{R_L \to \infty} \int_0^\infty \ln \frac{d\omega}{1 - |K(j\omega)|^2 \frac{4R_{o1}}{R_L}} \approx \lim_{R_L \to \infty} \int_0^\infty |K(j\omega)|^2 \frac{4R_{o1}}{R_L} d\omega \leqslant \frac{2\pi}{R_L C_{12}}$$

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Thus, we have

$$\int_0^\infty |K(j\omega)|^2 d\omega \leqslant \frac{\pi}{2R_{01}C_{12}} \tag{3}$$

This is the limiting condition of R-∞ network voltage transmission coefficient K toward the product of the network's output end capacitance C_{12} and input end resistance R_{o1}. We must derive the limiting condition of the network input end capacitance C_{o1}, but it is hard to use an integral like formula (3) to represent it at this time. Youla's theory can be of assistance. The main point of Youla's theory of R-R networks is that it describes a specific transmission coefficient function (such as the maximum flatness or Chebyshev response), allowing one to calculate a load resistance to satisfy this characteristic. To aid in visualizing this, we can set the internal resistance of the network power source $R_{o1} = 1$ and the response high-end frequency $\omega_{max} = 1$, and represent the load capacitance by the normalized value C'. At this time, the normalized device capacitance C_{o1} must be smaller than C' for it to be absorbed by network M'. If we assume that the maximum transmission coefficient response is 1, the actual value of C' should equal the normalized component values for the two ends of the low-pass prototype filter.

Now, we can first use the corresponding left end normalized capacitance C' in an R-R network having a response G = $|s_{21}(j\omega)|^2$, and then use network theory to derive the corresponding left end normalized capacitance C" for an R-∞ network which has the above equivalent response of $|K(j\omega)|^2$. Then we can derive the relationship between C' and C" for comparison, and we can use the limiting condition for C' to extrapolate the limiting condition for C".

First, we derive the equation to represent C' in an R-R network. At this time the S parameter which serves as the complex frequency s function can be expressed as

$$S_{11}(s) = \frac{h(s)}{g(s)} \quad S_{12}(s) = \frac{f(s)}{g(s)} \quad S_{22}(s) = \pm \frac{h(-s)}{g(s)} \tag{4}$$

In the formula, h(s), f(s), and g(s) are polynomial functions of complex variable s. Based on the relationship between the S parameter and the normalized resistance parameter, the z parameter can be expressed as

$$z_{11}(s) = \frac{g_e(s) + h_e(s)}{g_0(s) - h_0(s)} \tag{5-a}$$

$$z_{12}(s) = z_{21}(s) = \frac{f(s)}{g_0(s) - h_0(s)} \tag{5-b}$$

$$z_{22}(s) = \frac{g_e(s) - h_e(s)}{g_0(s) - h_0(s)} \tag{5-c}$$

The "e" and "0" shown in the formulas represent, respectively, the even and odd portions of the polynomial function.

For a low-pass network, $f(s) = 1$, so actually

$$G(j\omega) = |S_{21}(j\omega)|^2 = \frac{1}{g(s)g(-s)}\bigg|_{s=j\omega} \qquad (6)$$

From the network resistance we have

$$1 + h(s)h(-s) = g(s)g(-s) \qquad (7)$$

Formula (7) shows that polynomials $h(s)$ and $g(s)$ have similar maximum counts, and that the two should have equal maximum absolute coefficient values.

According to network theory, for the usual maximum flatness and Chebyshev response, the $h(s)$ zero distribution characteristics cause the maximum count of $h(s)$ to come when n is an even number and $h(s)$ is an even function, and when n is an odd number and $h(s)$ is an odd function, thus:

$$\left.\begin{array}{ll} h(s) = h_e(s) & n = \text{Even number} \\ h(s) = h_0(s) & n = \text{Odd number} \end{array}\right\} \qquad (8)$$

Given the characteristics of network M', both the front and tail ends are parallel capacitances, n should be an odd number, and the highest counts of $h(s)$ and $g(s)$ are odd counts and are equal. Now, formula (5-a) can be converted to

$$\frac{1}{z_{11}(s)} = -\frac{g_0(s) - h_0(s)}{g_e(s) + h_e(s)} = \frac{g_0(s) - h_0(s)}{g_e(s)} \qquad (9)$$

Because the maximum coefficient absolute values of $g(s)$ and $h(s)$ are equivalent, and because Cauer method network synthesis was used in formula (9) to suggest that the first resistance should be a parallel capacitance, we can give opposite signs to the maximum coefficient equivalents of $g_0(s)$ and $h_0(s)$ to assure that the degree number of the numerator in formula (9) is one degree higher than the denominator. We set the highest coefficient of $g_0(s)$ at G_n and the first coefficient of $g_e(s)$ at G_{n-1}, thus using the Cauer method to suggest that the first normalized capacitance would be:

$$C' = \frac{2G_n}{G_{n-1}} \qquad (10)$$

Now, reconsidering an R-∞ network, it also is expressed in normalized form, as shown in Figure 4. C" and C''' are, respectively, the front and tail end normalized parallel capacitances. The limiting condition for C''' was derived in formula (3) (and expressed as C_{i2} in the formula). Now we must derive the limiting condition for C".
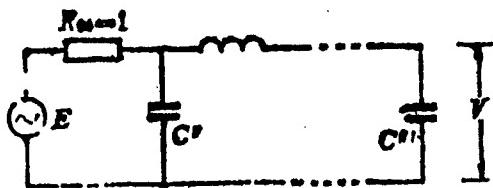
Figure 4. Normalized Form for an R-∞ Network

To distinguish it from an R-R network, the normalized resistance parameter of network M' is represented by z', so the voltage transmission coefficient $K(s) = \dfrac{V}{E}$ in the diagram can be represented by parameter z':

$$K(s) = \frac{z'_{12}(s)}{1 + z'_{11}(s)} \tag{11}$$

If we make $|K(j\omega)|^2$ completely identical to the $G(j\omega)$ response in the R-R circuit, then based on formula (6) K(s) should be expressed as

$$K(s) = \frac{1}{g(s)} \tag{12}$$

However, g(s) is a network function of an R-R network, so the z'_{12} and z'_{11} in formula (11) should be expressed as a g function and h function, but they both correspond to an R-∞ network, so a distinction can be made using a ' symbol. Combining formulas (11) and (12), we can derive

$$\frac{1}{g'_0(s) - h'_0(s) + g'_e(s) + h'_e(s)} = \frac{1}{g(s)} \tag{13}$$

Thus, we have

$$\left. \begin{array}{l} g'_0(s) - h'_0(s) = g_0(s) \\ g'_e(s) + h'_e(s) = g_e(s) \end{array} \right\} \tag{14}$$

And finally we have

$$\frac{1}{z'_{11}(s)} = \frac{g'_e(s) - h'_0(s)}{g'_e(s) + h'_e(s)} = \frac{g_0(s)}{g_e(s)} \tag{15}$$

Comparing formulas (15) and (9), we can determine that when $|K(j\omega)|^2$ in an R-∞ network and $G(j\omega) = |S_{21}(j\omega)|^2$ in an R-R network have equivalent responses, the first normalized parallel capacitance suggested for an R-∞ network is

$$C'' = \frac{G_n}{G_{n-1}} \tag{16}$$

We can determine that it is exactly one-half of C' in an R-R network. Because we already have a table of normalized components for networks with various responses, we can assume that one-half have C'' as the front end capacitance of network M' to calculate the tail end capacitance from formula (3). We obtained the following network front and tail end

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normalized capacitances for different ripple Chebyshev responses when n = 3 and n = 5.

Table 1.   Table of Normalized Capacitances for Both Ends of a Chebyshev
Response R-∞ Network

In-band ripple (dB)	n = 3		n = 5	
	Front-end capacitance	Tail-end capacitance	Front-end capacitance	Tail-end capacitance
0.1	0.5158	1.0895	0.5734	1.3759
0.2	0.6137	1.1900	0.6697	1.4356
0.5	0.7981	1.3465	0.8509	1.5388
1.0	1.0118	1.5088	1.0674	1.6652

Table 1 shows that the front and tail end capacitances required for a R-∞ network are about 1:2, not equal. This ratio roughly equals the ratio between output capacitance and input capacitance in most microwave FET devices. Thus, network M' can absorb almost all component capacitance at both ends, and thereby effectively uses the gain bandwidth potential of the device. In an R-R network, the network cannot effectively absorb component capacitance because its front and tail end capacitances are the same and because the device's output/input capacitance ratio does not match, which affects its gain bandwidth product. Estimates are that for each inter-stage network, the gain of an R-∞ network is 6 dB higher than an R-R network of identical bandwidth. This is quite substantial in terms of the amplifier as a whole.

III.   Network Synthesis of Interstage Networks

The preceding analysis shows that an R-∞ low-pass network has a large gain bandwidth product for various actual microwave FET devices. When plotting component parameters, we can refer to the normalized capacitance table above in order to select an appropriate Chebyshev response function to make the ratio between the front and tail end capacitances of M' roughly equal to the ratio between the component's output and input capacitances. We assumed the above response of $|K(j\omega)|^2$ and made it equal to $G(j\omega)$, used formula (6) to derive the polynomial function g(s), assumed that the normalized input parameter $\dfrac{1}{z_{11}'(s)}$ for an R-∞ network derived in formula (15) was used to derive its even part and odd part, and then derived a normalized resistance component using the Cauer method as a continued fraction.

$$\frac{1}{z_{11}'(s)} = C_1's + \cfrac{1}{L_2's + \cfrac{1}{C_2's + \cfrac{1}{L_4's + \cdots\cdots}}} \tag{17}$$

In it, C_1', L_2', C_3', L_4'... are, respectively, the normalized parallel capacitance and series inductance values in an R-∞ network. When we know the high-end frequency f_{max} of the amplifier and the internal resistance of the network power source (actually, it is the component output resistance R_0), we can reduce the previous normalized values to true capacitance and inductance values. However, we must note that the front and tail end normalized capacitances of M' should be reduced into the device's output and input capacitances. Thus, under conditions plotted for f_{max}, the network's internal resistance should be determined from this. It usually is less than the component output resistance R_0, so a parallel resistance should be added at the output end of an interstage network to make the total resistance equal the required network internal resistance. Thus, an interstage network is composed of a resistive low-pass network with an added resistive component, and belongs to the "lossy matching" category.

The inductance and capacitance of an interstage network can be achieved by using centralized parameters or by using semi-centralized parameters with a high resistance line and low resistance line segment.

IV. Experimental Results

We used the above theory and design steps to make a 10 MHz to 2 GHz four-stage microwave FET ultra-broadband amplifier. First, after approximating the small signal S parameter derived from microwave FET testing, we prepared the simplified equivalent circuit in Figure 1. Then the plotted input/output capacitances and output resistance served as a basis for using network synthesis methods to calculate the interstage network and then achieve it using semi-centralized parameters. Based on S parameter data for this type of network and an actual FET, we calculated the gain response of a four-stage amplifier, shown by the dashed line in Figure 5. It has the embryonic properties of ultra-broadband response, indicating the correctness of the basic theory and network synthesis steps proposed in this article and the feasibility of using a simplified model for approximate representation of microwave FET characteristics. To compensate for errors in modeling the FET component, optimization methods can be used for local readjustment of matching network component parameters. The final gain response is indicated in the figure by a solid line, with slight improvement over the original results.

A polytetrafluoroethylene fiber plate was used as a basal plate to make sample microband-type amplifiers. To obtain a smaller input/output SWR, a parallel resistance of appropriate value was added at the input and output ends of the amplifier. The results of measurements of gain and of input and output SWR characteristics of the sample amplifiers are shown in Figure 6. The similarity between the design and measured results and the excellent input/output SWR characteristics are apparent. By choosing suitable final stage components, the 1 dB compression point power of the amplifier can be as high as 16 dBm.
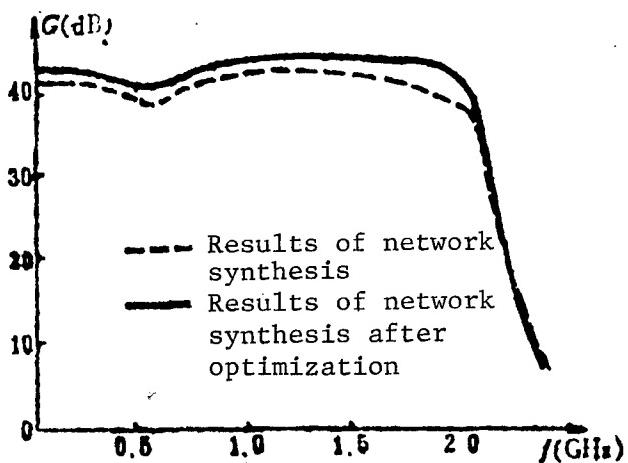
Figure 5. Comparison of Amplifier Gain Characteristics for Network Synthesis and After Further Optimization
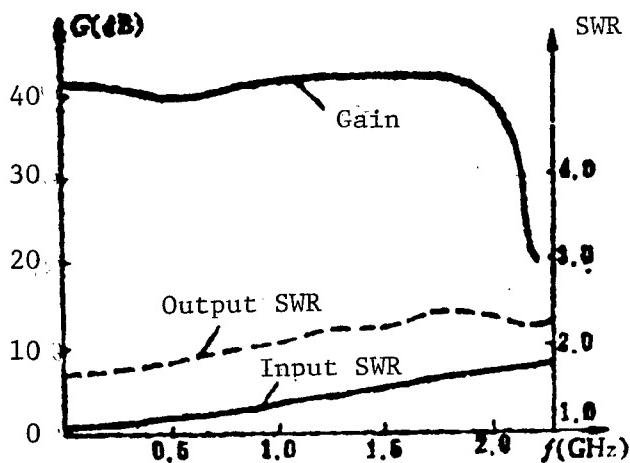
Figure 6. Measurement Results for a Sample Amplifier

V. Conclusion

We have suggested a microwave FET ultra-broadband amplifier circuit structure and the corresponding network synthesis methods. An R-∞ low-pass network was selected for interstage networks of the amplifier. Theory confirmed that this could give the amplifier a greater gain bandwidth product than other circuit structures. We fully exploited microwave FET device potential, and we established interstage network synthesis steps to push the achievement of matching networks beyond the chance and empirical design state in existing references. Similarly, a system design was made for an amplifier with resistant matching of the usual bandwidth. This can facilitate prediction of amplifier indices from component parameters, and the interstage network parameters derived from network synthesis can provide initial values for further optimization.

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The 10 MHz to 2 GHz sample ultra-broadband amplifier we made on the basis of the above theory and design steps showed excellent amplifier performance, and there was extremely close correspondence between the measured results and theoretical calculations, proving the correctness of the theory and design methods proposed in this article.

References

1. Kazuhiko Honjo, et al., IEEE Transactions on MTT, Vol 29, No 7, 1981, p 629.

2. Kazuhiko Honjo, et al., IEEE Transactions on MTT, Vol 30, No 7, 1982, p 1027.

3. K. B. Niclas, IEEE Transactions on MTT, Vol 30, No 11, 1982, p 1900.

4. H. W. Bode, Network Analysis and Feedback Amplifier Design, Van Norstrand, New York, 1945.

5. D. C. Youla, IEEE Transactions on C.T., Vol 11, No 3, 1964, p 30.

Design of Ka-Band Balanced Finline Mixer

[Article by Sun Daoli [1327 6670 4409] and Chen Zhenzhong [7115 2182 0022] of the Harbin Institute of Technology Microwave Teaching and Research Section; manuscript received March 1987]

[Text] Abstract

This article describes a new Ka-band finline balanced mixer and its circuit design. With functions similar to a waveguide mixer, it has the advantages of simple circuitry, ease in processing, broad working frequency, and so on. With a core of using only Chinese-made soft substrates and mixer diodes, we obtained excellent performance: a 36.5 GHz frequency up-conversion loss of 5.1 dB, a 3 dB bandwidth of 1500 MHz, and a signal port VSWR [voltage standing wave ratio] of less than 2.0.

I. Introduction

Finlines are new transmission lines used in millimeter-wave integrated circuits. A finline circuit is a planar printed circuit installed on the E surface of a rectangular waveguide. A finline has excellent properties and is structurally suited to applications in millimeter-wave hybrid integrated circuits[1]. Finline technologies are an important topic in millimeter-wave technology and are attracting growing attention.

Presently, the more successful millimeter-wave mixers are composed of a waveguide, finline, and microstrip and Schottky beam-lead diode. It can be stated that low-cost automated production of millimeter-wave systems in the past few years has depended mainly on microwave hybrid integrated circuit technologies based on finline technology[2].

II. Circuit Description of the Ka-Band Finline Balanced Mixer

Figure 1 is a circuit diagram for the Ka-band finline balanced mixer we developed. The local oscillator energy passes through the waveguide into microstrip transition conversion and is applied to the two diodes by a one-quarter wavelength

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coplanar line. A waveguide-to-finline transition device is used for the radio frequency input. A balanced finline and unbalanced coplanar line, which are two kinds of orthogonal-mode transmission lines (the main mode in the finline is H_{10} while the main mode in the coplanar line is a quasi-TEM), are interconnected to form a 180° power dividing circuit, and two diodes are soldered onto it. The signals in the two diodes have identical phases but reverse local oscillator phases. This forms a local oscillator reverse-phase balanced mixer. The series-connected finline concave strip on the signal channel is used to charge the broadband matching network between the diode junction capacitor and signal port. The intermediate frequency signal is transmitted from the unbalanced end of the line through a 50 Ω microstrip line. A broadband isolation coupler composed of a single-link one-quarter-wavelength coupled microstrip line segment in the local oscillator channel is used to isolate the local oscillator and intermediate frequency.

Figure 1.

The entire circuit was printed on a 0.3-mm-thick polytetrafluoroethylene woven-glass-fiber Chinese-made soft substrate (εr = 2.80). We used an 8-mm gallium arsenide Schottky mixer diode tube core produced by the Changchun Semiconductor Plant instead of the beam-lead diode used in finline hybrid integrated circuits. Figure 2 [not reproduced] is a photograph of this mixer.

III. Circuit Design of the Ka-Band Finline Balanced Mixer

1. Design of the mixer tube base

The mixer we designed is a broadband-operating one, and is a mirror image matched mixer. The effects of the diode's parasitic parameters can be ignored temporarily to derive the approximate design formula of the dual-tube balanced mixer during broadband operation[2].

Optimum frequency conversion loss
$$L_{copt} = \frac{1 + g_2/g_0}{(g_1/g_0)^2}\left(1 + \sqrt{1 - \frac{2(g_1/g_0)^2}{1 + g_2/g_0}}\right) \qquad (1)$$

Optimum input conductance and output conduction

$$G_{sopt} = \frac{1}{2}\, g_0\left(1 + \frac{g_2}{g_0}\right)\sqrt{1 - \frac{2(g_1/g_0)^2}{1 + g_2/g_0}} \qquad (2)$$

$$G_{oopt} = 2g_0\sqrt{1 - \frac{2(g_1/g_0)^2}{1 + g_2/g_0}} \qquad (3)$$

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In the formulas, g_o, g_1, and g_2 are the Fourier coefficients for single tube mixer diode junction conduction

$$\begin{cases} g_1/g_0 = B_1/B_0 \\ g_2/g_0 = B_2/B_0 \end{cases} \quad (4)$$

$$B_n(x) \xrightarrow{x = aV_p \gg 1} \begin{cases} \dfrac{e^x}{\sqrt{2\pi x}} \left[1 - \dfrac{4n^2 - 1}{1!\,8x} + \dfrac{(4n^2 - 1)(4n^2 - 3)}{2!\,(8x)^2} \cdots \cdots \right] & 0 \leqslant \arg x \leqslant \pi \\[3mm] \dfrac{e^x}{\sqrt{2\pi x}} \left[1 - \dfrac{4n^2 - 1^2}{1!\,8x} + \dfrac{(4n^2 - 1^2)(4n^2 - 3^2)}{2!\,(8x)^2} \cdots \cdots \right] & -\pi \leqslant \arg x \leqslant 0 \end{cases}$$

With this, it is very easy to derive the following group of design data:

$I_s(\mu A)$	$I_{d1}(\text{mA})$	$V_p(V)$	g_0	g_1	g_2	$Z_{sopt}(\Omega)$	$Z_{oopt}(\Omega)$	$L_{copt}(\text{dB})$
10	1.5	0.172	0.0604	0.0558	0.0441	170.23	73.70	3.53

Based on Z_{sopt} and Z_{opt} we can determine the structural dimensions of the finline and coplanar line which form the mixer tube base.

2. Signal and local oscillator channel

Theoretical analysis and experimental comparisons were done for seven common finline transition devices. We felt that the optimum shape for the finline transition device was an exponential line and sinusoidal line[3]. A sinusoidal line shape was used for transitional conversion of the radio frequency input end waveguide to the (single) finline transition device and for the local oscillator input end waveguide to bipolar finline transition conversion.

Considering the effects of the diode's parasitic parameter (junction capacitance), a series-connected finline concave band ahead of the diode pair forms the finline circuit diode broadband matching circuit shown in Figure 3. In it the optimum-shaped sinusoidal line shape is used for the finline transition device. We can derive the design dimensions of the finline concave band based on the design formula for the finline concave band non-continuous equivalent circuit and the broadband matching network between the two capacitative resistances[3].
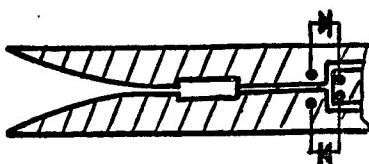
Figure 3.

A broadband isolation coupling circuit in the local oscillator channel is achieved by a single link one-quarter wavelength coupling microstrip line. See appendix B in note [2] for its design process.

IV. Performance of the Ka-Band Finline Mixer

During circuit development, we first used photoetching techniques to make a circuit diagram enlarged 10 times. Next we used a microscopic camera to reduce it the same number of times to make an extremely-high-precision circuit negative, and then made a soft substrate using printed circuit etching techniques. The diode core was sintered onto the circuit and installed into a prepared waveguide fixture to form a complete finline mixer.

Altogether we made five such circuits. Figure 4 shows the characteristic curves for those which performed the best.
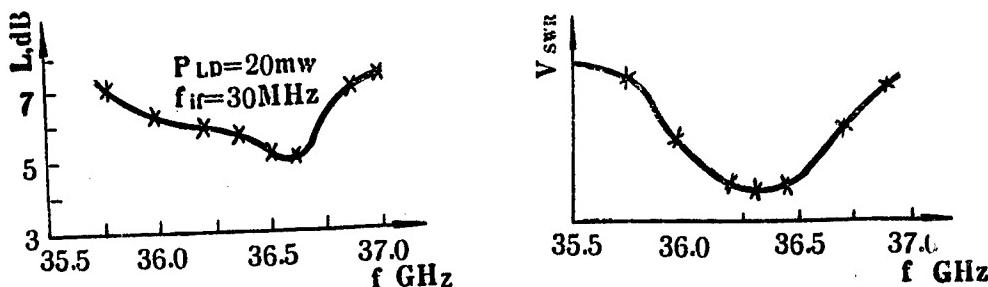
Figure 4.

V. Conclusion

The performance of the Ka-band finline balanced mixer was similar to that of a waveguide mixer, and it has the advantages of structural simplicity, broad working frequency, and so on. No DC bias voltage was configured to simplify the circuit, so this mixer has substantial local oscillator power. In addition, there was a problem with using a diode tube core instead of a beam-lead diode. This was circuit asymmetry, and led to rather small isolation (25 dB) between the local oscillator and signal port. Because of restrictions by conditions, mixer noise coefficients were not measured. We can make a rough estimate of them based on the above formula

$$F = t_d(L - 1) + 1 \qquad\qquad (5)$$

In it, F is the mixer noise coefficient, L is the frequency conversion loss, and t_d is the mixer diode signal-to-noise ratio.

References

1. K. Socbach, The Status of Printed Millimeter-Wave E-Plane Circuits, IEEE Transactions on MTT, Vol MTT-31, No 2, pp 107-121, February 1983.

2. Chen Zhenzhong [7115 2182 0022], Research on Ka-Band Finline Balanced Mixers, Harbin Institute of Technology Masters Thesis, July 1987.

3. Sun Daoli [1327 6670 4409] and Chen Zhenzhong, Design of Schottky Diode Bases in Finline Circuits, Paper at 1987 National Microwave Symposium, June 1987.

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